A CHLOROPLAST PHYLOGENY OF AGAVACEAE SUBFAMILY CHLOROGALOIDEAE WITH A FOCUS ON SPECIES RELATIONSHIPS IN HASTINGSIA

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CHAPTER I

INTRODUCTION

SERPENTINE SOILS

"Serpentine" is a general term used to encompass all ultramafic rocks and the soils that develop from them, as well as the biotic communities they support. Ultramafic rocks are unique substrates in that their chemical composition is unlike the majority rocks found on the continental crust. Instead, ultramafic rocks are more geologically similar to the sub-oceanic crust from which they originated. At spreading centers in the ocean, liquid magma from the Earth's mantle seeps out and cools to become new oceanic crust. As an oceanic plate collides with a continental plate, the denser oceanic plate is subducted under and recycled back into the mantle. However, as subduction is occurring, portions of the oceanic plate are accreted onto the continental plate, leaving embedded slabs of ultramafic rocks (Coleman 1977). Over time, suites of plants have become specially adapted to inhabit the distinctive, but harsh conditions found on these serpentine soils developed from ultramafic outcrops.

. The unique chemistry of serpentine soils creates difficult growing conditions for most plants. Plant growth is limited by high concentrations of iron and magnesium, which are often found at concentrations greater than 70% in the form of ferro-magnesium silicates (Brady et al.

2005). Calcium, on the other hand, is limited in serpentine soils, and in the presence of high magnesium concentrations, it becomes even less available to plants (Brady et al. 2005). The low Ca^{2+}/Mg^{2+} ratios are arguably the biggest influence on plant growth and species composition in serpentine plant communities (Alexander et al. 2007), but there are many other characteristics of serpentine that create edaphic communities. Heavy metals such as chromium, copper, lead, and nickel are found at concentrations toxic to most plant species. Additionally, serpentine soils are generally poor in other essential nutrients (N, K, P), and are often accompanied by erosion and poor water retention.

Most plants have adapted over time to the lighter elements that compose the majority of soils developed from the continental crust. However, many plants have adapted to tolerate the heavier elements that make up ultramafics. Plants can tolerate the conditions of serpentine soils in a number of ways. High concentrations of heavy metals can be tolerated by converting the excess metals into non-toxic compounds by the use of chelating agents or by excluding them from entering the plant's tissues (Gabbrielli et al. 1990; Tilstone and Macnair 1997). Low levels of calcium, on the other hand, are tolerated by either selective uptake of calcium, keeping normal Ca^{2+} levels within the plant cells, or by tolerating low levels within the plant cells (Marrs and Proctor 1976). However, the adaptive mechanisms that allow plants to survive soils with high concentrations of heavy metals and low Ca^{2+}/Mg^{2+} ratios are still poorly understood (Brady et al. 2005).

Although serpentine soils are most notable for their distinctive chemical composition, they are also accompanied by high levels of erosion and poor water retention (Alexander et al. 2007). Drought tolerance is arguably as important for serpentine inhabitants as the ability to tolerate low Ca^{2+}/Mg^{2+} ratios and high levels of heavy metals. In a study among serpentine tolerant and serpentine intolerant races of *Mimulus guttatus,* Hughes *et al.* (2001) found that drought tolerance was more highly correlated with other habitat characteristics than tolerance of low Ca^{2+}/Mg^{2+} ratios. Similar studies have shown that drought tolerance was more advantageous

than heavy metal tolerance to plants growing on serpentine soils (Freitas and Mooney 1995; Chiarucci 2003). However, not all serpentine sites are dry and barren. Serpentine fens and riparian areas can remain wet and lush throughout the whole year. For example, serpentine fens and seeps support many rare, endemic species that are not found in the drier surrounding chaparrals and savannas, like the carnivore, *Darlingtonia californica,* or the orchid, *Cypripedium californicum*. Despite the lush appearance, serpentine fens are still subject to high concentrations of heavy metals, low Ca^{2+}/Mg^{2+} ratios, and low nutrients, but also represent a small proportion of serpentine habitats.

With the number of stresses that serpentine soils present, one might expect serpentine outcrops to be depauparate of plant life in general. In some cases this is true: serpentine soils often have less vegetation than the surrounding non-serpentine soils. However, the number of species unique to serpentine soils is tremendous. Within California alone, at least 13% of all endemic species are endemic to serpentine substrates (Kruckeberg 1984), and of all rare, threatened and endangered species, 17% are limited to serpentine (Skinner and Pavlik 1994). These are particularly large percentages, given that serpentine outcrops represent a mere 1.5% of California's total land area (Harrison et al. 2000). There are well over 50 plant families containing taxa that are associated with serpentine in California alone (Safford et al. 2005). Despite this evident diversity, the mechanisms by which plants begin to inhabit and eventually become endemic to serpentine soils are poorly understood.

Several workers, like Stebbins (1942), Stebbins and Major (1965), and Kruckeberg (1954; 1957) have proposed two general mechanisms by which species become serpentine endemics: 1) species start out widely distributed and over time become restricted to serpentine habitats through multiple independent adaptations (depleted or paleoendemic species), or 2) the serpentine species arise from a single origin from a few pre-adapted individuals in a neighboring non-serpentine population (insular or neoendemic species). In a study of *Streptanthus glandulosus* (Brassicaceae), a species found mostly on serpentine, Kruckeberg (1954; 1957)

found evidence in support of Stebbins's paleoendemic hypothesis. Kruckeberg determined that there were at least two serpentine biotypes, and interpreted this as evidence for the possible existence of many biotypes that have been reduced to mainly serpentine tolerant ones. In a chloroplast DNA phylogenetic study of this species complex, Mayer and Soltis (1994) found further evidence that these were paleoendemics. The phylogeny suggested multiple origins of serpentine tolerance, with non-serpentine populations most closely related to serpentine populations that were geographically proximal. Gene flow between the populations was considered, but evidence from allozyme, interfertility, and morphological studies suggested that chances of hybridization were very low. There are other examples of similar patterns, such as that presented in a study of a close relative of *Streptanthus*, *Caulanthus amplexicaulis* (Pepper and Norwood 2001), but also in more distantly related taxa like *Allium* (Nguyen et al. 2008), and *Calochortus* (Patterson and Givnish 2004). In each case, serpentine endemics arose from non-serpentine populations, with no evidence that serpentine populations in turn give rise to non-serpentine populations.

In a very large-scale study of serpentine endemics of California from 23 different genera, Anacker et al. (2010) tested for directional biases in the evolution of serpentine endemism. Like the studies mentioned before, they found that in many cases serpentine endemism arose independently within genera, and transition rates off of serpentine tolerance were significantly lower than transitions producing serpentine tolerators and endemics. They also tested for changes in diversification rates and found lower rates of diversification along branches leading to serpentine endemic taxa than the non-serpentine taxa. They hypothesized that the consequence of becoming specially adapted to a narrowly distributed habitat is a loss of genetic diversity, preventing these endemic populations from further diversification.

The patchy nature of exposed serpentine rock creates many small and isolated populations, leading in many cases to extreme rarity and endemism. The consequences of small population sizes are numerous and potentially detrimental. Genetic drift and inbreeding depression become

increasingly problematic as population size decreases, causing a loss of genetic diversity and increased homozygosity, which in turn increase the chance of extinction (Ellstrand and Elam 1993). Because of this, serpentine and similarly fragmented populations are more sensitive to disturbances than more widespread populations.

Chlorogaloideae

Chlorogaloideae sensu Speta (1998) is a subfamily of Hyacinthaceae consisting of four genera. Many of the species inhabit serpentine soils or other poor soils of unusual chemistries, which provides a useful model for understanding how serpentine endemism evolves. However, understanding these evolutionary processes requires a solid understanding of relationships of the subfamily and among the constituent genera. Although the inclusion of *Chlorogalum* Kunth, *Schoenolirion* Torr ex Durand*, Hastingsia* S. Watson (formerly included in *Schoenolirion*)*,* and *Camassia* Lindl. in a distinct taxon is accepted by some treatments (Bentham and Hooker 1883; Cronquist 1981; Speta and Adler 1998), there have been no phylogenetic analyses that have explicitly investigated relationships within the subfamily nor has the monophyly of the group been examined. Recent phylogenetic studies have placed *Camassia* and *Chlorogalum* within Agavaceae (Bogler and Simpson 1996; Bogler et al. 2006; Alexander 2007; Smith et al. 2008), or alternatively within Agavoideae in the expanded Asparagaceae (APGIII 2009; Chase et al. 2009). However, no formal taxonomic treatment of Chlorogaloideae has been presented since Speta (1998). For the purposes of this paper, I will refer to the taxon consisting of *Chlorogalum, Camassia, Hastingsia* and *Schoenolirion* as "Chlorogaloideae sensu Speta", even though there is strong evidence for its placement within Agavaceae, rather than Hyacinthaceae, as proposed by Speta (1998). Notably, Speta acknowledged the possible placement of Chlorogaloideae within Agavaceae rather than Hyacinthaceae (p. 268). In this study, I examine the monophyly of Chlorogaloideae and relationships among the four genera. Examining these relationships

provides a basis for understanding when and how serpentine endemism arose within this group of monocots and contributes to our overall understanding of monocot systematics. Historically, the systematics of the monocotyledons has been problematic, particularly in Liliaceae, for which highly divergent taxonomic treatments have been presented, depending on the characters used to delineate taxa (Bentham and Hooker 1883; Krause 1930; Hutchinson 1959; Cronquist 1981; Dahlgren et al. 1985; Takhtajan 1997; Kubitzki and Huber 1998). Liliaceae sensu Cronquist (1981), whose concept is still widely used (for example, *Flora of North America,* 2002), treats the family as a large and heterogeneous group that is largely polyphyletic. Much work has been done to sort out these taxa based on phylogenetic evidence, but still many relationships remain poorly understood (Chase et al. 1995; Chase 2004; Chase et al. 2006; Graham et al. 2006; Chase et al. 2009).

Descriptions of the genera of Chlorogaloideae—The Chlorogaloideae sensu Speta consist of four genera endemic to North America. They are characterized as bulbous monocots with basal rosettes of long linear and keeled leaves, with the bulbs surrounded by a fibrous or membranous tunic. The flowers have superior ovaries with three fused carpels and arise from scapose racemes or panicles. Like members of Agavaceae, Chlorogaloideae share the cytological character of having a strongly bimodal karyotype, consisting of one set of large and one set of small chromosomes. A summary of selected characters that are variable among species of Chlorogaloideae and *Hesperocallis* A. Gray are presented in Table 1.

Chlorogalum consists of five species ranging from southwestern Oregon, throughout California, and into northern Baja California (Jernstedt 2002). They have paniculate inflorescences with small white to pink flowers (blue to purple in *C. purpureum* Brandegee), and some species have leaves with undulating margins. There are two morphological species groups within the genus: those with diurnally opening flowers, and those that are vespertine (opening during the evening) (Hoover 1940). The vespertine species, *C. pomeridianum* (DC.) Kunth, *C. grandiflorum* Hoover, and *C. angustifolium* Kellogg, also differ from the diurnal species in that

the styles are shorter than or equal in length to the tepals. The widest ranging species is *C. pomeridianum,* which occurs as far north as Douglas and Coos counties of southwestern Oregon, and as far south as northernmost Baja California. The distribution of *C. pomeridianum* overlaps with that of *Camassia* and *Hastingsia* in southwestern Oregon and northern California. There are three described varieties, with *C. pomeridianum* var. *pomeridianum* being the most widely distributed from SW Oregon to southern California with some populations found on serpentine in southwest Oregon*, C. pomeridianum* (DC.) Kunth var. *divaricatum* (Lindl.) Hoover occurring on bluffs along the central California coast, and *C. pomeridianum* var. *minus,* which is restricted to serpentine soils of north and central California. *Chlorogalum pomeridianum* (DC.) Kunth var. *minus* Hoover is often mistaken for *C. grandiflorum,* which is found in central California along the west foothills of the Sierra Nevada and is also restricted to serpentine soils. *Chlorogalum angustifolium* is found in the Sacramento and San Joaquin valleys of central California, both on and off serpentine soils, and also in southwestern Oregon where it is restricted to serpentine soils. Of the diurnal species, *C. parviflorum* S. Watson is found in coastal sage scrub habitat in southwestern California and northern Baja California. *Chlorogalum purpureum* consists of two varieties, with *C. purpureum* var. *purpureum* occurring only in three to four populations in open woodlands of the Santa Lucia Mountains, and *C. purpureum* Brandegee var. *reductum* Hoover found in only two populations in the La Panza Range of the South Coast Ranges restricted to serpentine soils.

Camassia consists of six species, and ranges throughout the northwestern three-quarters of the US, reaching Alberta and British Columbia, Canada (Ranker and Hogan 2002). Species of *Camassia* differ from *Chlorogalum, Schoenolirion*, and *Hastingsia* (in part) in that the inflorescences are racemose with larger, showier flowers ranging from blue to purple or less commonly white. Each locule of the ovary contains 3 to many ovules, versus 1-2 in the rest of Chlorogaloideae. There are four western and two eastern species. Of the western species, *C. quamash* (Pursh) Greene is the most widespread, ranging from southern British Columbia and

Alberta, Canada, south to central California, and east to Wyoming and Montana. *Camassia leichtlinii* (Baker) S. Watson is the second most widespread species, with populations occurring in and west of the Cascades and Sierra Nevada from southern British Columbia to northern California. *Camassia howellii* S. Watson is a serpentine endemic known to occur only in the Illinois Valley of southwestern Oregon, sympatric with *Hastingsia*. The fourth western species, *C. cusickii* S. Watson occurs only in northeastern Oregon and adjacent Idaho. The two eastern species, *C. angusta* (Engelm. & A. Gray) Blank and *C. scilloides* (Raf.) Cory, occur in the Great Plains, Ozarks, and Appalachians, ranging from Ontario south to Texas and Georgia. The distribution of *C. scilloides* overlaps that of *Schoenolirion croceum* in the southeastern US (Sherman 1969).

Schoenolirion consists of three species in the southeastern US (Sherman 2002). The genus has small flowers like those of *Hastingsia,* with colors ranging from yellow to white. The most widely ranging species occupying the most diverse habitats is *S. croceum* (Michx.) Alph. Wood*,* which can be found as far west as the pine barrens of eastern Texas and western Louisiana, as far north as Tennessee and northern Alabama in limestone cedar glades or on sandstone and granitic outcrops, and as far east as southern Georgia and northern Florida along the edges of bald cypress swamps. *Schoenolirion wrightii* Sherman has the most restricted distribution of the three species. It occurs west of any *S. croceum* population in eastern Texas, occupying similar habitats, and also south of any *S. croceum* population in northern Alabama, on sandstone outcrops. Lastly, *S. albiflorum* (Raf.) R.R. Gates is distributed in the southeastern corner of Georgia and throughout most of Florida, occupying the edges of bald cypress swamps. *Schoenolirion* is unique among the Chlorogaloideae in having a vertical rhizome, although it is surmounted by a small tunicate bulb in *S. croceum* and *S. wrightii* only. While no species of *Schoenolirion* are found growing on serpentine soils, they are often found on poor soils of unusual chemistries (relative to surrounding environments) such as limestone, granite, sandstone, and chalk (Anderson et al. 1999).

Hastingsia consists of four species distributed on serpentine outcrops in the Klamath-Siskiyou Mountains of southwestern Oregon and northern California (Becking 2002). The inflorescences of *Hastingsia* are often racemose, but are sometimes moderately branching like the paniculate inflorescences of *Chlorogalum*. Flower color is typically white, but is deep reddish-purple in *H. atropurpurea*. *Hastingsia alba* (Durand) S. Watson is the most widely distributed species, ranging from the northern Illinois Valley in southwest Oregon to the southern Cascades and northern Sierra Nevada in California. A morphologically similar species, *H. serpentinicola* Becking, is thought to be sympatric with *H. alba* throughout most of its range, but does not reach as far southeast as the Sierra Nevada (Becking 1989; Becking 1993). *Hastingsia bracteosa* S. Watson and *H. atropurpurea* Becking are sympatric with *H. alba* and/or *H. serpentinicola*, but are strictly limited to a few creeks and *Darlingtonia* fens on the west side of the Illinois Valley in southwestern Oregon. These two species are largely parapatric, with *H. bracteosa* occurring to the north of *H. atropurpurea*, however a narrow zone of overlap has been noted (Lang and Zika 1997).

Taxonomic background—The history of classification for members of Chlorogaloideae has been variable, and is due in part to the historical problems in monocot systematics. In particular, Liliaceae has had highly divergent taxonomic treatments presented, depending on the characters used to delineate taxa (Bentham and Hooker 1883; Krause 1930; Hutchinson 1959; Cronquist 1981; Dahlgren et al. 1985; Takhtajan 1997; Kubitzki and Huber 1998). For example, Bentham and Hooker (1883) and Krause (1930) placed monocot taxa with superior ovaries like *Yucca, Hesperaloe,* and *Hesperocallis* in Liliaceae, while placing taxa with inferior ovaries, like *Agave* and *Polianthes,* in Amaryllidaceae. Cronquist (1981), on the other hand, emphasized vegetative characters, placing the more herbaceous taxa in Liliaceae, while placing arborescent or "woody" taxa in Agavaceae. In contrast, Dahlgren (1985), took a more holistic approach by incorporating many morphological, cytological, and even chemical characters to delimit liliaceous families, which resulted in placing members of Liliaceae in older treatments in several

orders and many, relatively small families. Currently, the Angiosperm Phylogeny Group (APG; 2009), which uses phylogenetic evidence to delimit taxa, recognizes Liliaceae as a relatively small family within the order Liliales, with more than half of the species formerly recognized in Liliaceae now belonging throughout families within the order Asparagales.

Chlorogaloideae genera were classified for many years in Liliaceae because of their non-specialized herbaceous monocot features. In Baker's (1872) early revision of tribes Chlorogaleae and Scilleae of Liliaceae, *Chlorogalum* was included in Chlorogaleae with paniculate, rather than racemose, genera like *Nolina* and *Bowiea,* while *Camassia* was placed in the tribe Scilleae. However, Watson (1879) placed *Schoenolirion, Hastingsia,* and *Chlorogalum* in the subtribe Chlorogaleae of the tribe Phlangieae, leaving *Camassia* outside of Chlorogaleae, but unplaced within the tribe Phlangieae. Chlorogaleae with the same circumscription as Watson (1879) was maintained as a subtribe, but placed within the tribe Asphodeleae by Bentham and Hooker (1883) and as subtribe Chlorogalineae in subfamily Asphodeloideae by Krause (1930), in both cases placing *Camassia* elsewhere: within the tribe Scilleae by Bentham and Hooker (1883) or subfamily Scilloideae by Krause (1930). However, both Small (1903) and Hoover (1940) concluded that genera of Chlorogaleae were more similar to *Camassia*, because of their mainly bulbous habit, than they were to other genera of Asphodeleae, which are mainly rhizomatous. Gould (1942) also stressed the morphological similarities among *Camassia, Schoenolirion* (including *Hastingsia*), and *Chlorogalum,* and noted similarities to *Hesperocallis*, a prescient hypothesis in light of recent phylogenetic work (Michael McKain, University of Georgia, unpublished data).

While consensus grew on the recognition of a taxon containing *Camassia, Schoenolirion, Hastingsia*, and *Chlorogalum*, family placement varied, but was increasingly ranked as subfamily Chlorogaloideae. The group continued to reside in a broad Liliaceae in some treatments (for example Cronquist 1981), but was more commonly placed in Hyacinthaceae (Dahlgren et al. 1985; Takhtajan 1997; Speta and Adler 1998). However, Speta (1998) noted these genera

represented an "alien element" within Hyacinthaceae and suggested a closer affinity to members of Agavaceae, but retained placement in Hyacinthaceae. At this time, there was growing evidence for the placement of Chlorogaloideae in Agavaceae. Cytological studies by Cave (1974) showed that *Chlorogalum* and *Camassia* had a strongly bimodal karyotype (consisting of one set of large and one set of small chromosomes) similar to many members of Agavaceae. This relationship was subsequently supported by phylogenetic analyses of DNA sequences (Bogler and Simpson 1996; Pfosser and Speta 1999; Bogler et al. 2006; Good-Avila et al. 2006; Smith et al. 2008), but sampling included at most 1-3 representatives of *Chlorogalum* and *Camassia* (Smith et al. 2008). In a phylogenetic study of *Camassia* including 45 accessions (Fishbein et al. 2010), only one representative of *Chlorogalum* and two (for the first time) of *Hastingsia* were included, showing a close affinity of the three genera, but the monophyly of the subfamily, the relationships among the genera, and its placement in Agavaceae were beyond the scope of the study, as more distant outgroups were not included.

Although molecular evidence supports the placement of Chlorogaloideae taxa in Agavaceae, its exact placement is unresolved, as are many relationships within the family. Historically, many members of Agavaceae have been placed within Liliaceae or Amaryllidaceae. For example, Bentham and Hooker (1883) placed superior-ovaried taxa like *Yucca* and *Dasylirion* in Liliaceae tribe Dracaeneae, but placed inferior-ovaried taxa like *Agave* in Amaryllidaceae subfamily Agavoideae. Hutchinson (1959) and Cronquist (1981) placed an emphasis on the "woody" habit instead of ovary position and subsequently placed taxa like *Agave, Beschorneria, Furcraea, Hesperaloe, Manfreda, Yucca, Nolina, Dasylirion,* and *Dracaena* in Agavaceae. Evidence for a unique bimodal karyotype, with 5 large and 25 small chromosomes, shared by *Agave* and *Yucca,* spurred a more conservative view of the family (McKelvey and Sax 1933; Whitaker 1934; Sato 1935; Granick 1944). Consequently, Takhtajan (1997) kept only the taxa with a bimodal karyotype like *Agave, Beschorneria, Furcraea, Hesperaloe, Manfreda, Prochnyanthes* and *Yucca.* When a bimodal karyotype was discovered

for both *Hesperocallis* and *Hosta*, Cave (1948) suggested that both genera are more similar to *Agave* and *Yucca* than to members of tribe Hemerocallideae of Liliaceae, in which they were placed at that time (Krause 1930). Traub (1972) emphasized the alliaceous scent of *Hesperocallis* and placed it in its own family Hesperocallaceae in the order Alliales. However, in light of similarities between *Hesperocallis* and *Hosta*, Dahlgren, Clifford and Yeo (1985) placed *Hesperocallis* and *Hosta* in Hostaceae.

Since the 1990's, phylogenetic analyses have supported the exclusion of woody taxa without the bimodal karyotype from Agavaceae, such as *Nolina, Dasylirion, and Dracaena* (Bogler and Simpson 1995; Bogler and Simpson 1996; Bogler et al. 2006; Smith et al. 2008). These analyses also support the placement of non-woody, herbaceous taxa with bimodal karyotypes such as *Hosta*, *Hesperocallis, Camassia* and *Chlorogalum* in Agavaceae. However, their phylogenetic placement relative to *Agave, Beschorneria, Furcraea, Hesperaloe, Manfreda,* and *Yucca* are largely unresolved. Interestingly, *Yucca* was found to be polyphyletic, with *Yucca whipplei* segregated as the separate genus, *Hesperoyucca* (Hanson 1993; Bogler and Simpson 1995). Smith et al. (2009) found *Hesperoyucca* and *Hesperaloe* to be more closely related to *Chlorogalum* and *Camassia* than to *Yucca, Agave, Beschornia, Furcraea,* and *Manfreda,* although bootstrap support for these relationships were low.

Recently, whole chloroplast genomic sequences of *Hesperocallis* (Michael McKain, University of Georgia, unpublished data) have been shown to be more similar to those of *Camassia* than to members of other major clades of Agavaceae. Likewise, the floral and vegetative morphology of *Hesperocallis* is very similar to members of Chlorogaloideae, especially the fibrous tunicate bulb, keeled leaves with undulating margins (like some *Chlorogalum*), and occasionally branching racemes. *Hesperocallis* A. Gray (1865) is a monotypic genus with the sole species, *H. undulata* A. Gray. Compared to members of Chlorogaloideae, it has relatively large and showy white flowers and a perianth that is tubular at the base. It is found in the Mojave and Sonoran Deserts of southern California, Nevada, Arizona, Baja California, and

northwestern Sonora, in dry, sandy flats (Utech 1993). Although the distribution does not overlap with any species of Chlorogaloideae, it approaches the ranges of *Chlorogalum pomeridianum* and *C. parviflorum* in southern California and Baja California, but is restricted to more arid environments. In light of this evidence, the relationship of *Hesperocallis* to members of Chlorogaloideae is investigated in this study.

The most recent treatment for Agavaceae has been to reduce the family to subfamilial rank, Agavoideae, within an expanded Asparagaceae (APGIII 2009). However, no new formal treatment for Chlorogaloideae since Speta has been proposed. Although all four genera are currently placed in subfamily Agavoideae by the APG (Chase et al. 2009), their placement within Agavoideae is unresolved. Additionally, only *Camassia, Chlorogalum,* and most recently *Hastingsia* (Fishbein et al. 2010) have been included in any phylogenetic analysis. Consequently, the monophyly of the group has not been evaluated, nor have relationships among the genera been investigated.

Species Circumscription in *Hastingsia*

Hastingsia contains two to four rare species that are typically restricted to isolated serpentine outcrops within the Klamath-Siskiyou region of Oregon and California, with one species ranging south to the northernmost Sierra Nevada. The first documented encounter of *Hastingsia* was by Henry Pratten in the summer of 1851 during an expedition to northern California (Durand 1855). Specimens were collected along Deer Creek near Nevada City, in the northern Sierra Nevada. Durand described Pratten's northern California specimen as *Schoenolirion album,* a new species within an established genus. Watson (1879) proposed that *S. album* be segregated as the type of a new genus, *Hastingsia.* Although generally well accepted, *Hastingsia* and *Schoenolirion* continued to be considered congeneric by some treatments (Krause 1930; Hutchinson 1959). In 1991, Sherman and Becking (1991) published a study outlining the major differences between the two genera. They concluded that the number of differences were

significant enough to warrant the segregation of the genera. Since then, *Hastingsia* has been recognized as a distinct taxon in all major treatments.

Shortly after *Hastingsia alba* was described, a second species of the genus, *H. bracteosa,* was found on Eight Dollar Mountain in southwestern Oregon by Thomas Howell in 1884 and described by Watson (1884). Watson argued that the discovery of *H. bracteosa* supported the distinctiveness of the genus from *Schoenolirion*, and noted that *H. bracteosa* was morphologically distinct from *H. alba. Hastingsia bracteosa* differs from *H. alba* by having a campanulate perianth, much shorter, included stamens, more prominent bracts, and larger, more glaucous leaves. In contrast *H. alba* has a perianth of reflexed tepals, exerted stamens, smaller bracts, and narrower, greener leaves. To date, the segregation of these two species has not been disputed.

Until the 1980's, only two species of *Hastingsia* were recognized. However, two additional species have been proposed by Rudolf Becking (1986, 1989) and are recognized in the *Flora of North America* (Becking 1993): *H. serpentinicola* is a segregate from *H. alba* and *H. atropurpurea* is a segregate from *H. bracteosa.* Recognition of these segregates has been questioned due to difficulty in consistently observing the diagnostic differences between the species, most of which pertain to the size of leaves and inflorescences, and, in the case of *H. atropurpurea*, the color of the perianth. Consequently, *H. serpentinicola* and *H. atropurpurea* are not recognized as species in some treatments, such as the Oregon Flora Project (Cook and Sundberg 2011), or by the USDA (www.plants.usda.gov).

According to Becking (1989), the distributions of *H. alba* and *H. serpentinicola* are sympatric throughout the Klamath-Siskiyou ranges of southwestern Oregon and northwestern California, but only *H. alba* occurs in the northernmost Sierra Nevada and the southernmost Cascades. Becking distinguished *H. serpentinicola* as a smaller plant than *H. alba,* occurring mainly on well drained and open hillsides of serpentine rock that are wet in the spring, but drying in early summer. In contrast, he considered *H. alba* to be more "robust" and found in or near

permanently wet fens, but not limited to serpentine. In Becking's treatment for *Hastingsia* in the *Flora of North America* (Becking 1993) and in his publication outlining the distinctions between *Schoenolirion* and *Hastingsia* (Sherman and Becking 1991), characters used to distinguish *H. alba* from *H. serpentinicola* are largely continuous, with overlapping ranges for stem and leaf length, leaf width, scape length, the degree and position of tepal reflexion, whether or not anthesis occurs while anthers are exerted, and whether or not they occur where water is available all year. Individuals with intermediate morphological characters and/or habitat are not uncommon, making it difficult to distinguish the two species using Becking's keys. For example, Becking's identifications of herbarium specimens are not consistent with respect to the degree of reflexion of the tepals, which are "rotate at about half of the tepal length" in *H. alba*, and "sharply reflexed fully at about 2/3 or more of their length" in *H. serpentinicola.* Although these characters are used in his keys along with multiple vegetative characters, several herbarium specimens with flowers that appeared to be rotate, rather than sharply reflexed, were identified by Becking, as *H. serpentinicola*. The *Jepson Manual of California* (McNeal 1993) uses the degree of reflexion in the tepals and whether the stamens are exerted past the tepals as the main characters to distinguish *H. alba* from *H. serpentinicola*. However, using this key versus Becking's key in the *Flora of North America* or in Sherman and Becking 1991 will result in different identifications, making Becking's interpretation for the distinction of the species ambiguous. For the purposes of identifying specimens for this study, I have chosen to use the key in the Jepson Manual, which emphasizes differences in perianth morphology, because of the highly overlapping nature of the vegetative characters.

Hastingsia bracteosa and *H. atropurpurea* are sympatric with both *H. alba* and *H. serpentinicola,* but are very narrowly restricted to creek banks and continually wet *Darlingtonia californica* fens of the adjacent hillsides of the Illinois River valley of southwestern Oregon (Becking, 1986). Becking separates *H. atropurpurea* from *H. bracteosa* by the presence of deep purple tepals and more glaucous leaves in the former species, as opposed to white tepals and

greenish leaves in the latter species. For the most part, their geographic distributions do not overlap, but several populations containing plants identifiable to both species, as well as pink-flowered individuals, have been documented (Lang and Zika, 1997; pers. obs.). Becking (1986) also compared morphological characters of these species, including dimensions of the bulbs, leaves, scapes, floral and inflorescence bracts, and raceme branches. He found the average measurements of these characters to be significantly different between the species. However, Lang and Zika (1997) were unable to consistently distinguish the species based on any character other than flower color. Some species in related genera are easily distinguished by flower color, such as *Schoenolirion croceum* and *S. wrightii,* but these pairs are also accompanied by differences in vegetative traits (Sherman, 1969). Lang and Zika (1997) were not convinced that differences other than perianth color segregated *H. atropurpurea* from *H. bracteosa* and proposed that the rank of *H. atropurpurea* be lowered to variety, i.e., as *H. bracteosa* var. *atropurpurea* (Becking) F. Lang & P. Zika.

Goals

In this study I use phylogenetic analyses of chloroplast DNA sequences to study the relationships among genera of the subfamily Chlorogaloideae and relationships within *Hastingsia.* My work addresses the following aims:

1) **Determine relationships among genera of the subfamily Chlorogaloideae.** Chlorogaloideae sensu Speta was originally a subfamily within Hyacinthaceae, but a wealth of evidence supports the placement of the genera in Agavaceae. However, evidence for this is based on phylogenetic studies that have included only *Chlorogalum, Camassia*, and *Hastingsia*, while *Schoenolirion* has never been sampled. Furthermore, the monophyly of the group, as well as relationships within it, have never been examined. Also, molecular evidence has indicated a

close relationship between *Hesperocallis* and *Camassia,* but no phylogenetic analysis has included *Hesperocallis* with members of Chlorogaloideae. Furthermore, serpentine tolerance is found in many of the species in Chlorogaloideae, but patterns of adaptation have not been evaluated. I will address the following questions:

   a. Do *Chlorogalum, Camassia, Hastingsia,* and *Schoenolirion* form a monophyletic group?

   b. Is *Schoenolirion* most closely related to *Hastingsia,* its former congener?

   c. What is the relationship of Chlorogaloideae to other members of Agavaceae?

   d. Has serpentine tolerance evolved once or many times within Chlorogaloideae?

2) Assess relationships within *Hastingsia.* Species delimitation within the genus has been problematic and consequently two of the four species are not currently recognized by some treatments. By constructing a chloroplast phylogeny of the genus with population level sampling of all four putative species of *Hastingsia*, I aim to assess relationships among evolutionary lineages within *Hastingsia,* evaluate current species circumscriptions, and determine population history within and among species. I will address the following questions:

   a. Do each of the four species of *Hastingsia* represent distinct, monophyletic lineages?

   b. Are *H. alba*/*serpentinicola* and *H. bracteosa/atropurpurea* each distinct lineages, regardless of individual species monophyly?

c. If no species is monophyletic, what are the major lineages in the genus?

d. What are the geographic patterns in *Hastingsia*?

The goal of this work is to resolve phylogenetic relationships among members of Chlorogaloideae. This work will provide a basis for understanding patterns of adaptations onto serpentine. In addition to the phylogenetic and evolutionary understanding gained by this project, the work will be beneficial in terms of conservation. Knowledge of the phylogenetic history of Chlorogaloideae and *Hastingsia* will add to our understanding of how fragmented populations evolve and may potentially benefit other populations undergoing fragmentations due to human disturbance. Furthermore, the characterization of inter- and intraspecific relationships within *Hastingsia* will inform conservation efforts related to rare and narrowly endemic taxa.

Table 1. Comparisons of selected characters for species of Chlorogaloideae and *Hesperocallis.*

Taxon	Rootstock	Leaves	Ovules per locule	Chromosome count	Occurring on serpentine
Camassia angusta	Bulb	Keeled	Three to many	n = 15	No
Camassia cusickii					No
Camassia howellii					Yes
Camassia leichtlinii					No
Camassia quamash					No
Camassia scilloides					No
Chlorogalum angustifolium	Bulb	Keeled	Two	n = 16	Yes
Chlorogalum grandiflorum				n/a	Yes
Chlorogalum parviflorum				n = 30	No
Chlorogalum pomeridianum var. pomeridianum				n = 15, 18	No
Chlorogalum pomeridianum var. minus				n = 18	Yes
Chlorogalum pomeridianum var. divaricatum				n = 18	No
Chlorogalum purpureum var. purpureum				n = 30	No
Chlorogalum purpureum var. reductum				n/a	Yes
Hastingsia alba	Bulb	Keeled	Two	n = 26	Yes
Hastingsia atropurpurea				n = 26, 27	Yes
Hastingsia bracteosa				n/a	Yes
Hastingsia serpentinicola				n/a	Yes
Schoenolirion albiflorum	Rhizome	Flat, terete, or slightly keeled.	Two	n = 12	No
Schoenolirion croceum	Rhizome and bulb			n = 12, 15, 16	No
Schoenolirion wrightii	Rhizome and bulb			n = 12	No
Hesperocallis undulata	Bulb	Keeled	N/A	n = 24	No

CHAPTER II

A CHLOROPLAST PHYLOGENY OF AGAVACEAE SUBFAMILY CHLOROGALOIDEAE

Introduction

Serpentine soils have been of great interest to ecologists and evolutionary biologists because they contain toxic concentrations of metals and low essential nutrients, giving rise to plant populations with high levels of endemism (Kruckeberg 1984; Brady et al. 2005; Alexander 2007). Serpentine soils are formed by the weathering of ultramafic rocks, which are characterized by low Ca^{2+}/Mg^{2+} ratios and high levels of heavy metals such as chromium, copper, lead, and nickel. Also, the patchy nature of exposed serpentine rock creates many small and isolated populations, leading in many cases to extreme rarity. A high density of serpentine outcrops is found in southwestern Oregon and northern California. Within California, serpentine endemics represent 13% of all rare, threatened and endangered taxa (Kruckeberg 1984; Safford et al. 2005), while serpentine outcrops represent a mere 1.5% of California's total land area (Harrison et al. 2000), making serpentine outcrops the site of incredible diversity. Fragmented populations, such as those inhabiting serpentine soils, are of special conservation concern due to the detrimental effects of genetic drift, inbreeding and higher probability of extinction (Ellstrand and Elam, 1993; Young et al. 2001). As a result, fragmented populations are more sensitive to human disturbances than more widespread populations. However, fragmentation may also be the impetus for speciation if isolated populations become genetically divergent and adapt to local conditions (Orr

and Smith, 1998). Serpentine endemics provide an important model for studying how fragmented species and populations evolve and may provide important insights into recently fragmented populations due to human disturbance.

Chlorogaloideae sensu Speta (1998) was most recently treated as a subfamily of Hyacinthaceae. The group consists of four genera occurring throughout North America with many species inhabiting serpentine soils or other poor soils of unusual chemistries, which provides a useful model for understanding how serpentine endemism evolves. *Chlorogalum* Kunth, *Schoenolirion* Torr ex Durand*, Hastingsia* S. Watson (formerly included in *Schoenolirion*)*,* and *Camassia* Lindl. were considered to comprise a distinct taxon in many treatments (Bentham and Hooker 1883; Cronquist 1981; Speta and Adler 1998); however, there have been no phylogenetic analyses that have explicitly investigated relationships within the subfamily nor has the monophyly of the group been examined. Recent phylogenetic studies and taxonomic works have supported the inclusion of these genera within Agavaceae (Bogler and Simpson 1996; Bogler et al. 2006; Smith et al. 2008; Fishbein et al. 2010), or alternatively within subfamily Agavoideae in an expanded Asparagaceae (APGIII 2009; Chase et al. 2009).

The genera of Chlorogaloideae are characterized as herbaceous monocots with basal rosettes of long linear and keeled leaves arising from bulbs surrounded by a fibrous or membranous tunic, with scapose racemes or panicles that support flowers with trimerous parts. Because of their general lilioid features, the genera of Chlorogaloideae were classified for many years in the broadly circumscribed Liliaceae (Baker 1872; Bentham and Hooker 1883; Krause 1930; Cronquist 1981), and also in Hyacinthaceae (Dahlgren et al. 1985; Takhtajan 1997; Speta and Adler 1998). However, evidence for a bimodal karyotype in *Chlorogalum* and *Camassia* (Cave 1974), indicates a close relationship to Agavaceae. The placement of *Chlorogalum* and *Camassia* within Agavaceae has been supported in numerous phylogenetic analyses (Bogler and Simpson 1996; Pfosser and Speta 1999; Bogler et al. 2006; Good-Avila et al. 2006; Smith et al. 2008). In all cases, the position of the genera within Agavaceae is unresolved. Furthermore,

Hastingsia has been sampled in only one study that examined relationships within *Camassia* (Fishbein et al. 2010), but *Schoenolirion* has not been sampled in any study, nor has the monophyly of the subfamily, or relationships among the genera been addressed. Additionally, *Hesperocallis* A. Gray is also an herbaceous monocot with keeled leaves arising from bulbs surrounded by a fibrous tunic, and a bimodal karyotype and was recently placed in Agavaceae in phylogenetic analyses (Pires et al. 2004; Bogler et al. 2006). Further, it appears to be closely related to *Camassia* in analyses of whole chloroplast genome data (Michael McKain, University of Georgia, unpublished data). Thus, the inclusion of *Hesperocallis* in Chlorogaloideae merits further investigation.

The goals of this study are to 1) investigate the monophyly of Chlorogaloideae, 2) investigate the monophyly of each of its genera and their relationships to each other, 3) determine the relationship Chlorogaloideae to other members of Agavaceae, and 4) examine the pattern of adaptation onto serpentine in Chlorogaloideae.

Methods

Taxon sampling— One to three individuals were sampled for each species of *Hastingsia, Chlorogalum, Schoenolirion, Camassia,* and *Hesperocallis,* with multiple samplings when possible to represent the geographic ranges for each species. In order to evaluate the monophyly of Chlorogaloideae and understand its relative position within Agavaceae, the outgroup taxa consisted of single accessions each of several Agavaceae sensu stricto genera selected to represent the major clades revealed in prior studies (Bogler and Simpson 1995; Bogler and Simpson 1996; Bogler et al. 2006; Smith et al. 2008): *Hesperoyucca whipplei* (Torr.) Baker, *Hesperaloe parviflora* (Torr.) J.M. Coult., *Agave shawii* Engelm., *Yucca glauca* Nutt., and *Hosta* Tratt., plus two distant members of Agavaceae sensu lato, *Leucocrinum montanum* Nutt. Ex A. Gray and *Eremocrinum albomarginatum* (M.E. Jones) M.E. Jones, often placed in the family Anthericaceae. Two additional and most distantly related taxa, *Asparagus officinalis* L.

(Asparagaceae sensu stricto) and *Dichelostemma capitatum* (Benth.) Alph. Wood subsp. *capitatum* (Themidaceae) were included in the outgroup for tree rooting. All of the above are placed in Asparagaceae sensu lato in the APGIII (2009) system.

Character Sampling— Genomic DNA was extracted from field collected and silica-dried leaf material or from herbarium specimens using a commercial kit (Wizard® Genomic DNA Purification Kit, Promega, Madison, Wisconsin or DNeasy® Plant Mini Kit, Qiagen, Valencia, California). Polymerase Chain Reaction (PCR) was used to generate templates for sequencing. Four plastid regions, the *rpl16* intron, and the *trnD–trnY–trnE–trnT, psbJ-petA,* and $trnS^{UGA}$-$trnfM^{CAU}$ intergenic spacers were amplified using "universal" primers or primers newly developed for this study (Table 3), when nonspecific amplification occurred or if genomic DNA was severely degraded in the case of some herbarium material. Reactions were carried out with the iCycler® or C1000® thermal cyclers (Bio-Rad Laboratories, Hercules, California). Amplicons were generated using a standard 50 or 25 µl reaction consisting of 1 µl of genomic DNA undiluted or diluted by a factor of 10–50, 0.5 mM of both forward and reverse amplification primers, 200 µM dNTPs, 1.5 mM $MgCl_2$, 1× reaction buffer supplied by the polymerase manufacturer, 5% DMSO, and 0.2 U *Taq* DNA polymerase (Promega). Difficult templates were amplified in similar reactions, for which HotMaster® *Taq* DNA polymerase (Eppendorf, Westbury, New York) and supplied buffer were substituted and DMSO was omitted. PCR reactions were conducted with the following cycling conditions: 30 or 35 cycles of 95°C for 1 min, 51-56°C for 1 min, 65°C for 4 min, followed by a final extension at 65°C for 8 min and final hold at 4°C. Amplicons were purified for DNA sequencing by column filtration (Wizard® SVGel and PCR Cleanup System, Promega). DNA sequences were obtained by direct cycle sequencing with ABI Prism® BigDye® Terminator v3.1 or 1.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, California) following the manufacturer's protocol. Unincorporated dye terminators were removed by centrifugation through columns of Sephadex™ G-50 Fine (GE Healthcare Bio-Sciences, Piscataway, New Jersey) or by ethanol/EDTA/sodium acetate precipitation following

the BigDye Terminator manufacturer's protocols. Dye-labeled fragments were visualized and analyzed on the ABI Prism® 3100 Genetic Analyzer (Applied Biosystems,) at the Oregon Health and Science University Sequencing Core or on the ABI 3730 DNA Analyzer (Applied Biosystems) at the Oklahoma State University Recombinant DNA and Protein Core Facility. Sequencing primers were selected to give complete double stranded coverage of each region to maximize accuracy and included both "universal" primers and new primers developed from an alignment of *Camassia*, *Hastingsia*, *Chlorogalum*, *Schoenolirion,* and *Leucocrinum* sequences. Complete sequences were assembled and edited with the SeqMan™II module of Lasergene ver. 6 (DNASTAR, Madison, Wisconsin).

Phylogenetic Analyses— Sequences were aligned by eye for each region with Se-Al ver. 2.0 (Rambaut 1996). In most cases gaps were easily interpreted as independent insertion or deletion (indel) events and were coded as binary or multistate characters in the alignment. However, regions containing ambiguously aligned sites were omitted from phylogenetic analyses.

Phylogenetic trees were inferred under the maximum parsimony (MP) and maximum likelihood (ML) criterion and by Bayesian inference (BI). All analyses were conducted on the concatenated sequences from the four different chloroplast gene regions with the assumption that recombination between the loci is unlikely to occur in the plastid chromosome. Two different alignments were used for the MP analyses: the "with indels" (WI) alignment in which indels coded as multistate characters were included and the "no indels" (NI) alignment in which no indels were included. MP trees (MPTs) were constructed using two different approaches, each on the WI and NI alignments. In the first approach, heuristic searches were implemented with PAUP* ver. 4.0b10 (Swofford 2002) and performed with 1000 replicates of stepwise random addition of sequences, holding one tree per step, followed by tree-bisection-reconnection (TBR), keeping a maximum of 100 trees of scores greater than or equal to one per replicate with 'MaxTrees' set to 10^7. Second, ratchet analyses (Nixon 1999) were implemented with PAUPRat v1 (Sikes and Lewis 2001) and PAUP* via the CIPRES Science Gateway v3.0 (www.phylo.org).

Twenty separate ratchet analyses were conducted with 200 rounds of reweighting each. For each round, 20% of the informative characters were reweighted, followed by a heuristic search with a single replicate of stepwise random addition of sequences, keeping one tree subject to TBR branch swapping. MPTs found in all 20 analyses were summarized with a single strict consensus tree. A total of four strict consensus trees were compared from the sets of MPTs generated from the heuristic and ratchet searches on the NI and WI alignments. Clade support for the MPTs was assessed for both alignments using nonparametric bootstrapping (BS; (Felsenstein 1985) and implemented in PAUP* with 5000 pseudoreplicates and 10 random-addition-starting sequences with trees subjected to TBR branch swapping, keeping a single tree for each pseudoreplicate.

ML trees were estimated on the NI alignment using the programs RAxML version 7.0.4 (Stamatakis 2006) and GARLI version 0.96 (Zwickl 2006). Tree estimation using RAxML was performed with the concatenated sequences partitioned by locus with the GTR+Γ (GTRGAMMA) model of nucleotide substitution assigned to each partition. Support values were obtained with RAxML by conducting 5000 pseudoreplicates utilizing the rapid bootstrapping algorithm (with the default of 25 GTRCAT rate categories). Tree estimation using GARLI was implemented without data partitioning, which is not an available option, and with the GTR+ Γ model of nucleotide substitution. Clade support using GARLI was obtained by conducting 100 (the maximum permitted) bootstrap replicates. Both the RAxML and GARLI analyses were implemented via the online CIPRES Portal.

BI was conducted using MrBayes, v3.12 (Ronquist and Huelsenbeck 2003) on the NI alignment. The optimal evolutionary model of nucleotide substitution for each cpDNA locus was chosen by applying hierarchical likelihood ratio tests (hLRTs) and the Akaike Information Criterion (AIC) as implemented in MrModeltest, version 2.2 (Nylander 2004). Independent substitution models for each of the cpDNA loci were employed by creating partitions in the concatenated dataset. Metropolis-coupled Markov chain Monte Carlo simulations were run with eight linked chains (seven heated and one cold) and default priors for all model parameters,

except the parameter controlling the temperatures of heated chains, which was reduced to 0.02. Two independent runs of 1 x 10^7 generations were compared to assess convergence to a stationary distribution of parameter samples by examining the average of standard deviations between the two runs of split frequencies in MrBayes and by examining the effective sample size (ESS) for each parameter using Tracer, version 1.4 (Rambout and Drummond 2009). A cut-off of 0.01 standard deviations and ESS's greater than 200 were used as guidelines to assess convergence of runs. After a burnin of 2.5 x 10^6 generations, parameter values (including trees) were sampled every 1000 generations from the stationary distribution to calculate posterior probabilities of parameters.

Hypothesis testing--- The Templeton (Wilcoxon signed-ranks;(1983), Winning-sites (Prager and Wilson 1988), and Shimodaira– Hasegawa (SH; (Shimodaira and Hasegawa 1999; Goldman et al. 2000) tests (implemented in PAUP) were used to evaluate whether constraining traditional and contemporary taxonomic groups to be (or not be) monophyletic resulted in significantly different topologies. A summary of the monophyletic constraints and test results are presented in Table 6. For the parsimony based Templeton and Winning-sites tests, heuristic searches (run under parameters used for the previous heuristic search) were performed on the WI and NI alignments under ten constraints for a particular monophyletic group. Strict consensus trees from the constrained searches were compared with those of the unconstrained searches to test for significant topological support. For the likelihood based SH test, I used GARLI to estimate the ML trees under the ten constraints on the NI alignment only, which were then compared to the unconstrained ML tree produced by GARLI. The SH-test was performed with the GTR + Γ model of nucleotide substitution using the resampling estimated log-likelihood method (RELL) (Kishino et al. 1990) with 1000 bootstrap replicates.

Ancestral character state reconstructions-- The "Database of Serpentine Endemism" of California Safford et al. (2005), Kruckeberg's (1984) monograph on serpentine endemism in California, the Jepson Manual (Hickman 1993), and numerous herbaria records (Consortium of

California Herbaria: http://ucjeps.berkeley.edu/consortium/) were used to characterize serpentine affinity for each taxon in this study. Taxa were identified as being serpentine tolerators, if the majority of occurrences have been observed on serpentine substrates, and serpentine nontolerators, if plants have never or rarely been observed to occur on serpentine. MP and ML ancestral state reconstructions were performed on the ML tree produced in GARLI using Mesquite 2.74 (Maddison and Maddison 2004). The ML reconstruction was performed under a single-rate Mk likelihood model for discrete morphological characters described by Lewis (2001). Unlike parsimony, the ML reconstruction considers branch lengths when estimating character states at a node. However, neither method considers topological uncertainty. Instead of using a single tree, stochastic character mapping (SM; Huelsenbeck and Bollback 2001; Huelsenbeck et al. 2003) can incorporate phylogenetic uncertainty by explicitly modeling branch length distributions in a Bayesian framework. In addition, Bayesian posterior probabilities of ancestral states can be assessed across a range of phylogenies, such as a sample from the posterior distribution of a Bayesian phylogenetic analysis. SM reconstructions were performed using the program SIMMAP v1.5 (Bollback 2006) with a single-rate 1/k model of evolution (Lewis 2001) on a sample of 1000 trees randomly chosen from the post-burnin posterior tree distribution from the Bayesian phylogenetic analysis.

Results

Sequence characteristics—The alignments for all four loci were easily accomplished by eye, although numerous short gaps were introduced. Due to sequencing difficulties, only partial sequences could be obtained for *Chlorogalum parviflorum, C. pomeridianum* SCA, and *Schoenolirion croceum* GA for the *trnD-trnT* spacer, and *Chlorogalum parviflorum* for the *rpl16* intron. In preliminary analyses, a lower percentage of variable sites in the *trnS-trnfM* spacer was found for congeneric taxa relative to the other three loci. Consequently, five accessions, representing additional samples for a particular species, were not sequenced for this region and

are missing from the multiple sequence alignments, including *Chlorogalum pomeridianum* SCA, *C. pomeridianum* var. *minus*, *Schoenolirion croceum* GA, *S. croceum* TN, and *S. wrightii* TX1. A total of 32 unambiguously aligned indels were coded as binary or multistate characters, with the number of coded indels ranging from 11 (*trnD-T*) to 5 (*trnS-fM*). Each of the four aligned regions was similar in terms of length and parsimony informative sites (Table 4).

Model selection— Best fitting models of evolution differed among the regions. For the *trnD-T* spacers, alternative model selection procedures preferred different models. All paths through the tree of hLRTs evaluated by MrModeltest resulted in the HKY + Γ model. Since the AIC implementation of MrModeltest also selected the GTR + Γ model, it was selected for use in the Bayesian analysis. For the *rpl16* intron, hLRTs preferred either the HKY + Γ or HKY + I, and AIC preferred HKY + Γ, which was selected for use. For the *psbJ-petA* and *trnS-fM* spacers, all hLRTs selected either the GTR + I or GTR + Γ models, while the AIC preferred GTR + Γ, which was selected for both regions. Table 5 presents the final parameter estimates of the chosen models.

Tree searches and support— MP, ML, and BI analyses produced phylogenetic estimates that were largely congruent, however, some relationships differed in resolution and congruence among tree estimation procedures. MP analyses for the conventional heuristic search and the ratchet search produced consensus trees identical in both length and topology for each alignment, with few differences in resolution between the NI and
WI alignments. The ML trees estimated in RAxML and GARLI and the 50% majority rule consensus of trees sampled from the stationary phase of the BI analysis were identical in topology, which is represented by the RAxML tree (Figure 3).

Chlorogaloideae (under all hypothesized circumscriptions) was found to be polyphyletic. The clade consisting of the genera of Chlorogaloideae sensu Speta (*Hastingsia, Camassia, Chlorogalum,* and *Schoenolirion*) also included *Hesperocallis, Hesperaloe,* and *Hesperoyucca* (Clade A; MP-WI BS = 85, MP-NI BS = 92, RAxML BS = 98, GARLI BS = 92, PP = 1; the

order of clade support values are maintained below). Within this clade, a subclade of *Hastingsia, Camassia,* and *Chlorogalum* was well supported as monophyletic (hereafter "core Chlorogaloideae"; 100, 100, 100, 100, 1), with *Hesperocallis* very weakly supported as sister to this clade (Clade B; <50, 0, 61, 62, 0.92). *Schoenolirion* was found in a strongly supported clade with *Hesperaloe* and *Hesperoyucca* (Clade C; 82, 92, 97, 96, 1), where a clade of *Schoenolirion* and *Hesperaloe* (97, 98, 99, 100, 1) was sister to *Hesperoyucca*. In all of the analyses, *Hastingsia, Camassia, Schoenolirion, Hesperocallis* were each highly supported as monophyletic (100, 100, 100, 100, 1), except *Chlorogalum,* which was found to be paraphyletic. All accessions of *Chlorogalum pomeridianum*, *C. grandiflorum,* and *C. angustifolium* ("core *Chlorogalum*") were placed in a strongly supported clade (100, 100, 100, 100, 1) that was moderately supported as sister to a *Hastingsia* and *Camassia* clade (74, 74, 82, 80, 1), but *C. purpureum* and *C. parviflorum* formed a grade or were unresolved with *Hastingsia, Camassia,* and core *Chlorogalum*. *Hastingsia* and *Camassia* were strongly supported as sister taxa (Clade E; 100, 100, 100, 100, 1). The accessions of *Hosta, Agave,* and *Yucca* formed a weakly supported clade (<50, <50, 62, 95, .97) that was sister to or were unresolved with clade A (99, 99, <50, 84, 0.99).

Within *Chlorogalum*, *C. pomeridianum* was not recovered as monophyletic. *C. grandiflorum* was found in the clade with all the *C. pomeridianum* accessions as sister to *C. pomeridianum CA* (66, <50, 66, 71, .96). However, all *C. angustifolium* accessions formed a moderately supported clade 54, <50, 72, 88, .92. Similarly, *Schoenolirion croceum* was not found to be monophyletic, with one accession, *S. croceum TN*, moderately supported in a clade with the *S. wrightii* and *S. albiflorum* accessions (54, 80, 94, 93, 1), but the three remaining accessions of *S. croceum* were weakly to moderately supported in a single clade (61, 64, 78, 66, .79).

Hypothesis testing—Results of the tests of taxonomic hypotheses are presented in Table 6. For Hypothesis 1a (Chlorogaloideae sensu Speta), optimal trees constrained to include a clade of *Hastingsia, Camassia, Chlorogalum,* and *Schoenolirion* differed significantly from unconstrained optimal topologies. Likewise, including *Hesperocallis* in the circumscription of

Chlorogaloideae (Hypothesis 1b) resulted in significantly less optimal trees for all tests except for both parsimony tests with the NI alignment. A third circumscription of Chlorogaloideae, suggested by the optimal trees for most analyses in this study, included *Hastingsia, Camassia, Chlorogalum,* and *Hesperocallis* (Hypothesis 1c). For the Templeton and WS test using the WI alignment and the SH test, optimal trees constrained to exclude this clade were significantly less optimal. Although the parsimony NI analyses did not recover this group as monophyletic, the unconstrained MP tree was not found to be significantly shorter than one in which this group was constrained to be monophyletic. Optimal trees in which the individual genera of *Hastingsia, Camassia,* and *Schoenolirion* were found to be monophyletic were all found to significantly differ from optimal trees that were constrained to exclude monophyly of each genus. *Chlorogalum* was found to be paraphyletic in all unconstrained analyses, but trees constrained to *Chlorogalum* monophyly were not found to be significantly worse. Topologies in which the clade of *Schoenolirion*, *Hesperaloe* and *Hesperoyucca* (Hypothesis 8) was constrained to be excluded were not significantly worse.

Ancestral character state reconstructions— Ancestral character state reconstructions of serpentine tolerance for the parsimony and likelihood reconstructions in Mesquite are presented in Figures 4 and 5. The most parsimonious reconstructions (MPRs) resulted in mostly unequivocal ancestral states, with five state changes required, whereas most likelihood and SM ancestral state assignments were less certain. Table. X presents likelihood and posterior probabilities (PP) from the SM analysis for selected nodes numbered in Figure 5. The ancestral node of the core Chlorogaloideae was reconstructed as intolerant of serpentine in the MPRs, and similarly, the likelihood and PP were only 0.27 and 0.37 that this ancestor occurred on serpentine. At the node including all core Chlorogaloideae except *C. parviflorum*, the MPR changes to serpentine tolerant. Serpentine tolerance at this node has a PP of 1.0, but a likelihood of only 0.35.

Similarly, the common ancestor of all core *Chlorogalum, Hastingsia,* and *Camassia* has an MPR of serpentine tolerance, with a PP of 1.0, but a likelihood of 0.38. Within this clade, the ancestral node for core *Chlorogalum* has a likelihood of occurring on serpentine of 0.50, similar to the common ancestor of *Hastingsia* and *Camassia* (likelihood = 0.45), while MPRs for both nodes are unequivocally serpentine tolerant, both with a PP of 0.93. The ancestral nodes of all *Camassia* and all *Camassia* species except *C. leichtlinii,* both have equivocal MPRs, and low likelihoods (0.11 and 0.01, respectively) and low PPs (0.015 and 0.0027) of serpentine tolerance. However, the *Hastingsia* ancestral node was estimated to be serpentine tolerant in the parsimony analysis, with a high likelihood and PP of 0.98 and 0.999, respectively.

Discussion

The non-monophyly of Chlorogaloideae— The monophyly of Chlorogaloideae sensu Speta was not supported. The clade consisting of *Chlorogalum, Camassia, Schoenolirion,* and *Hastingsia* also included *Hesperocallis, Hesperoyucca,* and *Hesperaloe* with moderate to high clade support (Fig. 1, 2, and 3). Although *Chlorogalum, Camassia,* and *Hastingsia* were recovered as monophyletic in all analyses, *Schoenolirion* consistently fell within a clade containing *Hesperoyucca* and *Hesperaloe*. Although the monophyly of Chlorogaloideae sensu Speta could be rejected statistically, a closer relationship of *Schoenolirion* to *Hesperoyucca* and *Hesperaloe* than to core Chlorogaloideae was not statistically significant (Table 6).

A close relationship of *Schoenolirion, Hesperoyucca,* and *Hesperaloe*, if true, is surprising and is contrary to over 100 years of systematic work (Baker 1872; Bentham and Hooker 1883; Krause 1930; Cronquist 1981; Dahlgren et al. 1985; Takhtajan 1997; Kubitzki and Huber 1998; Speta and Adler 1998; Pfosser and Speta 1999). In most phylogenetic studies,

Hesperaloe and *Hesperoyucca* were found to be sister groups, but their placement relative to other Agavaceae, such as *Camassia, Chlorogalum*, *Hosta, Yucca,* and *Agave* in the broadest sense (including *Beschorneria, Furcraea, Manfreda, Prochnyathes,* and *Polianthes*) is unclear because of low resolution or conflicting topologies (Bogler and Simpson 1995; Bogler and Simpson 1996; Bogler et al. 2006; Smith et al. 2008). In a chloroplast phylogeny of Agavaceae, Bogler and Simpson (1995) found *Hesperaloe* and *Hesperoyucca* as sister groups that fell outside of the major *Yucca* and *Agave* clade, either as this clade's only sister or also including *Hosta,* but this study did not include any members of Chlorogaloideae. One accession of *Camassia* was included in Bogler and Simpson's (1996) ITS phylogeny, but with conflicting results for its placement, depending on the partition of the data set. *Hesperaloe* and *Hesperoyucca* were recovered in a clade with *Yucca elata* and *Y. treculeana* in that study, but with low or no support, and *Camassia* was either placed as sister to a clade containing *Hesperoyucca, Hesperaloe,* the two *Yucca* species, and *Agave* (ITS1), was placed in a polytomy with these taxa (ITS2), or was weakly supported (56%) as sister to *Hesperoyucca, Hesperaloe,* and the two *Yucca* species (ITS1 and ITS2 combined). In a more densely sampled phylogenetic analysis of Agavaceae, Bogler et al. (2006) found conflicting results depending on whether the phylogeny was inferred from only chloroplast *ndhF* sequences, or a combined dataset of chloroplast *ndhF* and *rbcL* sequences and nuclear ribosomal ITS sequences. Chlorogaloideae (i.e., *Chlorogalum* and *Camassia*) was supported as either sister to a clade composed of *Hosta, Hesperoyucca, Hesperaloe, Yucca,* and *Agave* (*ndh*F) or *Hosta* was excluded and placed as sister to Chlorogaloideae and the remaining taxa above (*ndhF*, *rbcL*, and ITS combined). However, node support for these relationships was low (<63%).

While there are many similarities in morphology and geography between *Schoenolirion* and the core Chlorogaloideae, Sherman (1969) also describes many differences. For morphology, the most notable characters include a vertical rhizome that is found in all species of *Schoenolirion,* which terminates in a tunicate bulb in *S. croceum* and *S. wrightii*, but not in *S.*

albiflorum. However, no rhizome is present in any species of core Chlorogaloideae taxa. Notably, *Hesperaloe* and *Hesperoyucca* are both rhizomatous, similar to the remainder of Agavaceae, suggesting that the bulbs of *Schoenolirion* may have evolved independently from those of the core Chlorogaloideae. Similarly, all species of the core Chlorogaloideae have distinctly keeled leaves, whereas *Schoenolirion* has flat to abaxially rounded leaves, although the leaves are sometimes slightly keeled in *S. croceum* and *S. wrightii*. Also, the stigmas in *Schoenolirion* are described as entire to only slightly lobed, while the stigmas of the genera of core Chlorogaloideae are described as three lobed. *Schoenolirion* is found only in the southeastern US, reaching as far west as Texas and as far north as Tennessee and North Carolina. All species of the core Chlorogaloideae can be found within the Pacific coast states, from southern British Columbia to northern Mexico with only a few exceptions: *Camassia quamash* reaches as far east as western Montana, Wyoming, and parts of Utah, and *C. angusta* and *C. scilloides* are found only in the Midwestern to southeastern states, overlapping in distribution with *S. croceum* and *S. wrightii*. However, a chloroplast phylogeny of *Camassia* suggested that *C. angusta* and *C. scilloides* were derived from the western species, *C. quamash* (Fishbein et al. 2010), which did not provide a phylogeographic connection to *Schoenolirion*. The geographic distribution of *Hesperoyucca* is not sympatric with *Schoenolirion*, but is found in southern California, western Arizona, and Baja California. *Hesperaloe* occurs in closer proximity to *Schoenolirion*, in western and central Texas, south into northern Mexico, but does not overlap in range. A close relationship of *Schoenolirion* and *Hesperaloe* would suggest a southeastern U.S. origin for *Schoenolirion,* rather than a western origin in contrast to the core Chlorogaloideae.

The exact placement of *Hesperocallis* is unclear, but its possible placement as sister to the core Chlorogaloideae is intriguing. Except for the parsimony analysis of the NI alignment, *Hesperocallis* was found to be sister to the core Chlorogaloideae, but with weak support (Figs. 1, 2, and 3), but was statistically significant (Table 6). In the analyses of the NI alignment, the placement of *Hesperocall0is* is unresolved with respect to the core Chlorogaloideae and the clade

comprised of *Schoenolirion, Hesperoyucca,* and *Hesperaloe*. Similarly, the placement of *Hesperocallis* as sister to core Chlorogaloideae was not statistically supported in Bogler et al. (2006), in which it which it was resolved as sister to the rest of Agavaceae s.l. (*Hosta, Chlorogalum, Camassia, Hesperoyucca, Hesperaloe, Yucca,* and *Agave*). However, clade support separating *Hesperocallis* from the remainder of the genera was low in each analysis (<63%), and in addition no hypothesis tests were conducted, making their results inconclusive. Recently, a whole chloroplast genome sequence of *Hesperocallis* (Michael McKain, University of Georgia, unpublished data) has been shown to be more similar to that of *Camassia* than to members of other major clades of Agavaceae.

The floral and vegetative morphology of *Hesperocallis* is very similar to members of the core Chlorogaloideae. *Hesperocallis* has a fibrous and tunicate bulb as well as keeled leaves as in all core Chlorogaloideae, and undulating leaf margins similar to those found in *Chlorogalum*. However, compared to the core Chlorogaloideae, *Hesperocallis* has relatively large and showy white flowers and a shortly tubular perianth. It is found in dry, sandy flats of the Mojave and Sonoran Deserts of southern California, Nevada, Arizona, Baja California, and northwestern Sonora (Utech 1993). Although the geographic distribution of the sole species, *Hesperocallis undulata,* does not overlap with any species of Chlorogaloideae, it does approach the ranges of *Chlorogalum pomeridianum* and *C. parviflorum* in southern California and Baja California, but is restricted to more arid environments. Overall, the sister relationship of *Hesperocallis* to the core Chlorogaloideae is not well supported, however it is intriguing and should be investigated further with additional molecular data and morphological studies.

The core Chlorogaloideae—Although the relationships between the core Chlorogaloideae genera and other members of Agavaceae are not well resolved, many relationships within this clade were found to be well supported. The core Chlorogaloideae (*Chlorogalum, Hastingsia,* and *Camassia*) were supported as monophyletic with high bootstrap support and statistical significance in all analyses (Fig. 1, 2, and 3, Table 6). Further, the sister

group relationship of *Hastingsia* and *Camassia* was highly supported and statistically significant in all analyses. The idea of a sister relationship between *Camassia* and *Hastingsia* has been indirectly suggested in past taxonomic treatments. Initially, *Hastingsia* and *Schoenolirion* were thought to be most closely related, as indicated by their former congeneric status (Durand 1855), which was maintained in some later taxonomic treatments (Krause 1930; Hutchinson 1959). Watson (1879), however, who first distinguished *Hastingsia*, placed it with *Camassia, Chlorogalum,* and *Schoenolirion* in the tribe Phlangieae along with *Hesperanthes* (=*Echeandia*, now Anthericaceae), but placed only *Chlorogalum, Schoenolirion*, *and Hastingsia* in the subtribe Chlorogaleae. However, in Hoover's treatment for *Chlorogalum* (1940), he considered the genus to be equally related to both *Camassia* and *Schoenolirion* (including *Hastingsia*). Similarly, Gould (1942), suggested that *Camassia* was closely affiliated with *Chlorogalum* rather than *Hastingsia* or *Schoenolirion.* Alternatively, Sherman (1969) felt that *Camassia* and *Schoenolirion* were most closely related, stating that, "*Camassia* seems to represent a link between *Schoenolirion* and [*Hastingsia* and *Chlorogalum*]." The reason that previous authors have not explicitly predicted a sister relationship between *Camassia* and *Hastingsia* may be because there are no apparent synapomorphies uniting them. The most detailed morphological investigation of Chlorogaloideae sensu Speta was by Sherman in his unpublished Ph.D. dissertation (1969) on the systematics of *Schoenolirion.* He summarized morphological differences among the four genera, and concluded that *Camassia* differs from both *Chlorogalum* and *Hastingsia* by the presence of two to many ovules per locule versus only two ovules in the other taxa, whereas similarities shared by *Camassia* and *Chlorogalum* include the twisting of perianth segments together over the fruit when they dry (at least in some species of *Camassia*) versus the segments withering separately to the base in *Hastingsia*. Similarly, *Camassia* and *Chlorogalum* share the base chromosome number of $n = 15$, compared to $n = 26$ in *Hastingsia*. Both *Chlorogalum* and *Hastingsia* species more generally occur on serpentine substrates, whereas *Camassia* has only one species that occurs on serpentine. Similarly, biogeography is not particularly informative for

inferring relationships, with *Hastingsia* occurring at the northern end of the range of *Chlorogalum* and at the southern end of the range of *Camassia*. However, a close relationship to *Hastingsia* does add support for a Pacific Northwest origin for *Camassia* and *Hastingsia*, as hypothesized by Gould (1942) and further supported by Fishbein et al. (2010). However, a more detailed morphological investigation is needed in order to understand morphological diversification among the core Chlorogaloideae.

The non-monophyly of Chlorogalum— The genera *Hastingsia, Camassia, Schoenolirion,* and *Hesperocallis* were each sampled by multiple individuals and were recovered as monophyletic with high bootstrap support and statistical significance (Fig. 1, 2, and 3, Table 6). While *Hastingsia* (Chapter III) and *Camassia* (Fishbein et al. 2010) have been shown to be monophyletic in other analyses, the monophyly of *Schoenolirion* has never been tested previously. However, *Chlorogalum* was not supported as monophyletic in any analysis in this study. All accessions of *Chlorogalum pomeridianum, C. angustifolium,* and *C. grandiflorum* (the core *Chlorogalum*) formed a strongly supported clade (Fig. 1, 2, and 3), excluding the remaining species, *C. parviflorum* and *C. purpureum,* which were either paraphyletic or unresolved with respect to the remaining core Chlorogaloideae. However, the paraphyly of *Chlorogalum* was not statistically significant. Additionally, ML branch lengths uniting *C. parviflorum* and *C. purpureum* to the remainder of the core Chlorogaloideae are very short compared to the branch leading to the core *Chlorogalum,* suggesting that either a short amount of time or reduced substitution rates have occurred among the diverging lineages, indicating that rapid radiation may have occurred in the ancestral populations of the core Chlorogaloideae. Although the monophyly of *Chlorogalum* has not been previously questioned, a distinction between *C. parviflorum* and *C. purpureum* from *C. angusta, C. grandiflorum,* and *C. pomeridianum* has been noted. Most importantly, *C. parviflorum* and *C. purpureum* have diurnally opening flowers, while the other species are vespertine. The diurnal species also differ by the length of perianth segments, which are shorter than the styles. In the vespertine species, the perianth segments are equal to or longer

than the styles. Although the chloroplast-based phylogenetic results are intriguing, further investigations are necessary and should include additional independent loci (i.e., nuclear data) before any taxonomic revisions are undertaken.

Evolution of serpentine tolerance— Serpentine tolerance is found in a number of species of core Chlorogaloideae. Four species of *Chlorogalum* grow on serpentine soils at least in some populations or varieties (*C. purpureum* var. *reductum, C. angustifolium, C. grandiflorum,* and *C. pomeridianum* var. *minus*), in addition to one species of *Camassia* (*C. howellii*) and all species of *Hastingsia*. In the present study, the resolution of species relationships within the genera is not well supported, especially in *Chlorogalum,* in which not all species were recovered as monophyletic (i.e., *C. pomeridianum*) and not all varieties were represented. In the most parsimonious reconstruction, serpentine tolerance evolved once in the core Chlorogaloideae after divergence from *C. parviflorum*. Serpentine tolerance was maintained in the majority of remaining *Chlorogalum* species and in *Hastingsia*, but was lost in by some *Camassia* species. It is equally parsimonious to infer that serpentine tolerance was lost in the common ancestor of *Camassia*, but regained in *C. howellii,* or lost independently in *C. leichtlinii* and in the clade consisting of *C. quamash, C. angusta,* and *C. scilloides*, with *C. howellii* retaining ancestral serpentine tolerance. Similarly, the SM reconstructions suggest that the most probable ancestral state of all core Chlorogaloideae was serpentine intolerance, with the evolution of serpentine tolerance in this clade occurring after the divergence of *C. parviflorum*. Subsequently, tolerance was most probably maintained throughout *Chlorogalum* (with a reversal in *C. pomeridianum*) and *Hastingsia.* Serpentine tolerance was most probably lost in *Camassia* after diverging from *Hastingsia* and regained in *C. howellii*. In the likelihood reconstruction, ancestral serpentine tolerance of core Chlorogaloideae is less likely than intolerance, but the differences in likelihood are not great (the increasing probability of serpentine tolerance towards the base of the tree in the likelihood reconstruction is an artifact of fixing the root probability at 50%). Under the most likely scenario, tolerance evolved independently in each genus. This scenario is similar to

findings from other phylogenetic analyses of serpentine tolerant and endemic species, in which endemism arose many times within closely related species and genera (Mayer and Soltis 1994; Pepper and Norwood 2001; Patterson and Givnish 2004; Nguyen et al. 2008; Anacker et al. 2010). Within *Allium,* Nguyen et al. (2008) and Anacker et al. (2010) concluded that serpentine endemism arose at least five independent times, with few transitions out of the endemic state. Conversely, very few studies indicate that serpentine tolerance is maintained throughout multiple closely related genera as the parsimony and SM reconstructions suggest, the exception being serpentine tolerance within *Streptanthus* and *Caulanthus* (Mayer and Soltis 1994, 1999; Pepper and Norwood 2001). This may be an indication that this trait is not well studied at the generic level, or it may reflect the labile nature of serpentine tolerance, in which serpentine tolerance is often independently gained within taxa. The conflicting results from the parsimony, likelihood, and SM reconstructions are difficult to interpret with certainty. The likelihood reconstructions only consider a single tree when calculating likelihoods at ancestral nodes, whereas the SM analysis calculates the Bayesian posterior probabilities of ancestral states across a range of phylogenies. Therefore, the likelihood reconstructions may be less robust than the Bayesian based SM approach. Other fully Bayesian methods for reconstructing ancestral character states, such as BayesTraits (Pagel et al. 2004; Pagel and Meade 2006), should be considered as well.

Many species of *Allium* are well adapted to the well-drained Mediterranean climate found throughout California where species of *Allium* are often found growing on serpentine soils. This is perhaps evidence of the importance of drought tolerance for taxa inhabiting serpentine soils as indicated by several studies of serpentine tolerance (Freitas and Mooney 1995; Hughes et al. 2001; Chiarucci 2003). The genera of the core Chlorogaloideae are most closely related to genera within Agavaceae that are particularly drought tolerant and often occupy desert like climates (i.e., *Agave, Yucca, Hesperoyucca, Hesperaloe, Hesperocallis*). *Schoenolirion* is similarly adapted to such conditions. For

example, *S. croceum* occupies the shallow soil islands on granitic outcrops in the southeastern US, which undergo severe drought because of limited water-holding capacities, as well as extreme temperature changes (Anderson et al. 1999). The widespread adaptations to arid conditions in Agavaceae may confer a preadaptation to the well-drained and poor soils of serpentine outcrops for the core Chlorogaloideae.

Conclusions— The goals of this study were to examine the monophyly of Chlorogaloideae sensu Speta (1998), determine intergeneric relationships, and reconstruct the history of adaptation onto serpentine soils. A phylogeny of Chlorogaloideae was constructed from four chloroplast loci, in which the monophyly of *Schoenolirion, Chlorogalum, Camassia,* and *Hastingsia* (Chlorogaloideae sensu Speta) was not supported, with *Schoenolirion* being more closely related to *Hesperaloe* and *Hesperoyucca*. The monophyly of a clade consisting of *Chlorogalum, Camassia,* and *Hastingsia* (the core Chlorogaloideae) was well supported, as was a sister relationship of *Hastingsia* and *Camassia.* Although the placement of *Hesperocallis* was not fully resolved, the results from this study suggest a sister relationship to the core Chlorogaloideae. All but one of the individual genera were strongly supported as monophyletic, including *Schoenolirion, Camassia, Hastingsia,* and *Hesperocallis*, the exception being *Chlorogalum*, which was paraphyletic. The results from the ancestral character state reconstructions suggest multiple, independent adaptations onto serpentine within the Chlorogaloideae.

More data are clearly needed in order to further resolve relationships between the core Chlorogaloideae, *Hesperocallis*, *Schoenolirion, Hesperaloe*, *Hesperoyucca,* and their placement within Agavaceae. More chloroplast data may increase resolution or support for relationships that have also been elusive in other phylogenetic studies (Bogler and Simpson 1995; Bogler and Simpson 1996; Bogler et al. 2006; Smith et al. 2008). However, these analyses also have conflicting results depending on the loci used to infer the phylogeny. Conflicting topologies from independent loci may indicate that incomplete lineage sorting (ILS) occurred during the

diversification of these lineages. ILS, a phenomenon in which alleles assort independently in diverging populations, has been shown to create conflicting topologies among independent loci, especially in rapidly radiating taxa (Maddison 1997; Maddison and Knowles 2006; Carstens and Knowles 2007; Degnan and Rosenberg 2009). Additional molecular data are needed, but should be obtained from both nuclear and chloroplast genomes, to estimate the most accurate phylogeny for these taxa. Future investigations should also include a denser sampling of taxa within Agavaceae to estimate relationships more precisely. Additional taxa should also be sampled within *Chlorogalum* to investigate its lack of monophyly; in this study *C. parviflorum* and *C. purpureum* were only represented by a single accession each. Similarly, additional alternate ancestral character state reconstructions, especially those based on Bayesian inference such as BayesTraits (Pagel et al. 2004; Pagel and Meade 2006), which consider topological uncertainty should be compared to the parsimony, likelihood, and SM reconstructions for inferring serpentine tolerance in the core Chlorogaloideae. Additionally, a more densely sampled phylogeny, especially within *Chlorogalum* in which some taxa were recovered as para- and polyphyletic, should be used to reconstruct the history of serpentine tolerance.

Table 2. Population locations and vouchers for samples analyzed in this study.

Taxon name and population code	Location	Voucher
Agave shawii	Near El Rosario, Baja California, 30.08804˚N 115.65064˚W	Fishbein 6405 (OKLA)
Asparagus officinalis	Stillwater, OK. In cultivation.	Halpin 105 (OKLA)
Camassia angusta	Shelby County, MO, 39° 43.417'N 92° 10.150'W	Gremaud s. n. (WILLU)
Camassia cusickii	Hells Canyon, near Halfway, OR, 45° 03.468'N 116° 54.210'W,	Cronquist 6549 (OSC)
Camassia howellii	Sexton Mountain, near Grants Pass, OR, 42° 35.675'N 123° 22.268'W	Kephart 593 (WILLU),
Camassia leichtlinii	Popcorn Swale, near Glide, OR, 43° 18.079'N 123° 13.558'W	Kotaich 105 (WILLU)
Camassia quamash CA	Donner Lake, near Truckee,CA, 39° 19.356'N 120° 14.811'W	Sultany s. n. (WILLU)
Camassia quamash BC	University of Victoria, Victoria, British Columbia, 48° 27.658'N 123° 19.134'W	Allen 1310 (WILLU)
Camassia scilloides	Harms Woods, Glenview, IL, 42° 03.666'N 87° 46.200'W	Carlson s. n. (WILLU)
Chlorogalum angustifolium CA1	Near Redding, CA. N 42° 24'24.4" W 123° 00'21.5	Callahan s. n. (OSC)
Chlorogalum angustifolium CA2	Near Colfax, CA.	Hillaire 1246 (CHSC)
Chlorogalum angustifolium OR	Near Gold Hill, OR. N 40° 38' 30.80", W122° 21' 47.50"	Callahan s. n. (OSC 216206)
Chlorogalum grandiflorum	Near Chinese Camp, CA. N 37° 51' 35.50", W 120° 27' 36.40"	Callahan s. n. (OSC)
Chlorogalum parviflorum	Near Encinitas, CA . N 33.092 W 117.288	Sanders 30293 (UCR)
Chlorogalum pomeridianum OR	8 Dollar Mountain, near Selma, Oregon 42° 13.89'N 123° 39.06'W	Fishbein 5972 (OKLA)
Chlorogalum pomeridianum var. *minus*	Near Sunnyside, California. 39° 53' 50.60", -122° 58' 23.60"	Callahan s. n. (OSC)
Chlorogalum pomeridianum SCA	Near Sunland, CA. N 34.254 W 118.321	Gross 405 (CHSC)
Chlorogalum purpureum	Near Jolon, CA.	Wilken 15701 (SBBG)
Eremocrinum albomarginatum	Near Mexican Hat, San Juan Co., Utah, 37.19913˚N 109.87934˚W	Fishbein 6520 (OKLA)
Hastingsia alba CA1	Trinity Mountains, near Hayfork, California, 40° 21.678'N 123° 12.798'W	Halpin 6 (HPSU)
Hastingsia alba CA2	Lassen National forest, near Chester, California, N40°24.411' W121°21.763'	Halpin 20 (OKLA)
Hastingsia bracteosa	8 Dollar Mountain, near Selma, Oregon, 42°	Fishbein 5969 (HPSU)

	14.68’N 123° 40.95’W	
Hastingsia serpentinicola	Lone Mountain, near O’Brien, Oregon, 42°03.10'N 123°44.64'W	Fishbein 5932 (HPSU)
Hesperaloe parviflora	Stillwater, Oklahoma. In cultivation.	Fishbein (needs voucher)
Hesperocallis undulata CA1	Riverside County, Joshua Tree National Park, California. 33º54.602’N 115º50.130’W	Prince 520 (RSA)
Hesperocallis undulata CA2	Near Agua Caliente Springs, San Diego Co., California, 32.97479˚N 116.32734˚W	Fishbein 6470 (OKLA)
Hesperocallis undulata BCA	Near Bahía de los Angeles, Baja California, 29.03249˚N 113.82114˚W	Fishbein 6423 (OKLA)
Hesperoyucca whipplei	Near Ejido Uruapan, Baja California, 31.59477˚N 116.42676˚W	Fishbein 6390 (OKLA)
Hosta	Portland, Oregon. In cultivation.	Halpin 87 (OKLA_
Leucocrinum montanum	Lost Forest, near Christmas Valley, Oregon, 43° 21.847’N 120° 19.758’W,	Ruedas s. n. (HPSU)
Schoenolirion albiflorum	Near Fellsmere, FL, 27 47'10.9"N, 80 33'02” W	Scanlon 405 (FLAS)
Schoenolirion croceum TN	Cedars of Lebanon State Park, near Lebanon, TN.	Bailey s. n. (5/8/02) TENN
Schoenolirion croceum TX1	Near Burkeville, TX	Singhurst 6557 (TEX)
Schoenolirion croceum TX2	Near Kirbyville, TX	Holmes 11266 (TEX)
Schoenolirion croceum GA	Near Multrie, GA	Godfrey 76354 (FLAS)
Schoenolirion wrightii	Near Huntsville, TX	Keith 129 (BRIT)
Yucca glauca	McPherson Preserve, near Stillwater, OK	Halpin 88 (OKLA)

Table 3. Sequences of primers used for amplification and sequencing.

Primer	Sequence (5' to 3')	Source
rpl16-F71	GCTATGCTTAGTGTGTGACTCGTTG	Jordan et al. 1996
rpl16-R1661	CGTACCCATATTTTTCCACCACGAC	Jordan et al. 1996
rpl16-F608HAS	GATTCATCGGGTGGGATGGCGG	Fishbein et al. 2010
rpl16-R697HAS	GGGTTTCGCGGGCGGATATTG	Fishbein et al. 2010
trnD–F	ACCAATTGAACTACAATCCC	Shaw et al. 2005
trnT	CTACCACTGAGTTAAAAGGG	Shaw et al. 2005
trnE	AGGACATCTCTCTTTCAAGGAG	Shaw et al. 2005
trnY	CCGAGCTGGATTTGAACCA	Shaw et al. 2005
$trnS^{UGA}$	GAGAGAGAGGGATTCGAACC	Demesure et al. 1995
trnfM	CATAACCTTGAGGTCACGGG	Demesure et al. 1995
trnS-AGVF	GGATTCGAACCCTCGATATG	This study
trnfM-AGVR	CACGGGTTCAAATCCTGTCTC	This study
trnSfM-F520AGV	GGATTGGATTAGTCTTTCTGG	This study
trnSfM-R600AGV	AATGTGTCTCAYAATCCGC	This study
psbJ	ATAGGTACTGTARCYGGTATT	Shaw et al. 2007
petA	AACARTTYGARAAGGTTCAATT	Shaw et al. 2007
psbJpetA-AGVF	GAATTTGGATATGCGTAAAAATC	This study
psbJpetA-AGVR1	GACTTTGACTCTTTTGGTTG	This study
psbJpetA-AGVR2	GACTCTTTTGGTTGAAAAGCGG	This study

Table 4. Attributes of the aligned sequences of four plastid loci for all taxa including outgroups; values in parentheses exclude distant outgroups *Asparagus* and *Dichelostemma*.

Locus	Aligned length (bp)	Length after ambiguously aligned regions excluded (bp)	Variable sites included (bp)	Parsimony informative sites included (bp)	Indels and inversions coded as multistate characters
trnD-trnT	1367	1025	218 (157)	111 (93)	11
rpl16 intron	1517	947	170 (116)	82 (71)	9
psbJ-petA	1556	1041	205 (141)	98 (85)	7
trnS-fM	1384	1203	177 (130)	82 (80)	5
Total	5824	4216	770 (544)	373 (329)	32

Table 5. Parameter estimates of substitution models selected for use in Bayesian and likelihood analyses.

Partition	Model	T_i/T_v	r_{AC}	r_{AG}	r_{AT}	r_{CG}	r_{CT}	π_A	π_C	π_G	π_T	α
Parameters for Bayesian analysis – 95% credible interval excluding burn in values.												
trnD-trnT	GTR+Γ	-	0.286-0.331	0.257-0.381	0.035-0.080	0.049-0.135	0.241-0.363	0.298-0.351	0.158-0.201	0.154-0.197	0.295-0.348	0.366-1.048
rpl16 intron	HKY+Γ	2.566-4.571	-	-	-	-	-	0.353-0.408	0.132-0.173	0.171-0.216	0.247-0.299	0.433-14.556
psbJ-petA	GTR+Γ	-	0.089-0.177	0.262-0.389	0.028-0.067	0.039-0.141	0.191-0.308	0.323-0.378	0.140-0.181	0.130-0.169	0.312-0.367	0.460-1.845
trnS-fM	GTR+Γ	-	0.156-0.274	0.220-0.357	0.033-0.082	0.034-0.132	0.202-0.330	0.288-0.337	0.161-0.200	0.148-0.186	0.315-0.365	0.307-1.192
Parameters for likelihood analysis in RAxML												
trnD-trnT	GTR+Γ	-	0.901	2.644	0.465	0.639	2.435	0.31	0.19	0.19	0.31	0.522
rpl16 intron	GTR+Γ	-	0.449	1.57	0.520	0.331	2.158	0.38	0.16	0.20	0.27	0.820
psbJ-petA	GTR+Γ	-	1.153	2.808	0.398	0.681	2.171	0.34	0.16	0.16	0.34	0.689
trnS-fM	GTR+Γ	-	1.271	1.799	0.329	0.434	1.594	0.31	0.18	0.17	0.34	0.286
Parameters for likelihood analysis in Garli												
4-loci combined	GTR+Γ	-	1.005	2.322	0.444	0.579	2.241	0.34	0.17	0.17	0.32	0.592

Figure 1. Strict consensus of all equally most parsimonious trees discovered by conventional heuristic and ratchet searches analyzed from the NI (no indels coded) dataset. Nonparametric bootstrap percentages greater than 50% presented above branches.

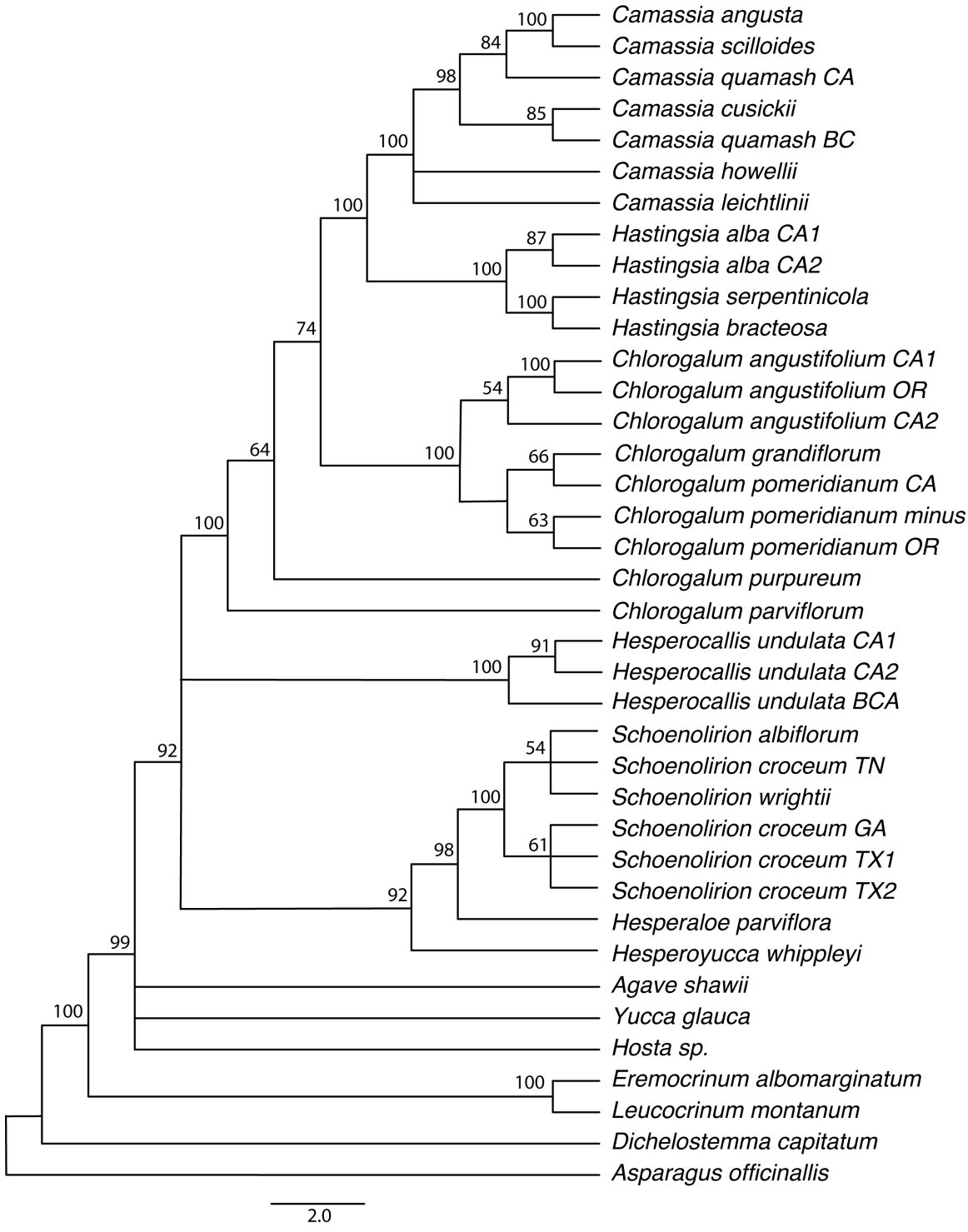

Figure 2. Strict consensus of the equally most parsimonious trees discovered by conventional heuristic and ratchet searches analyzed with the WI (with indels) dataset. Nonparametric bootstrap percentages greater than 50% are presented above branches.

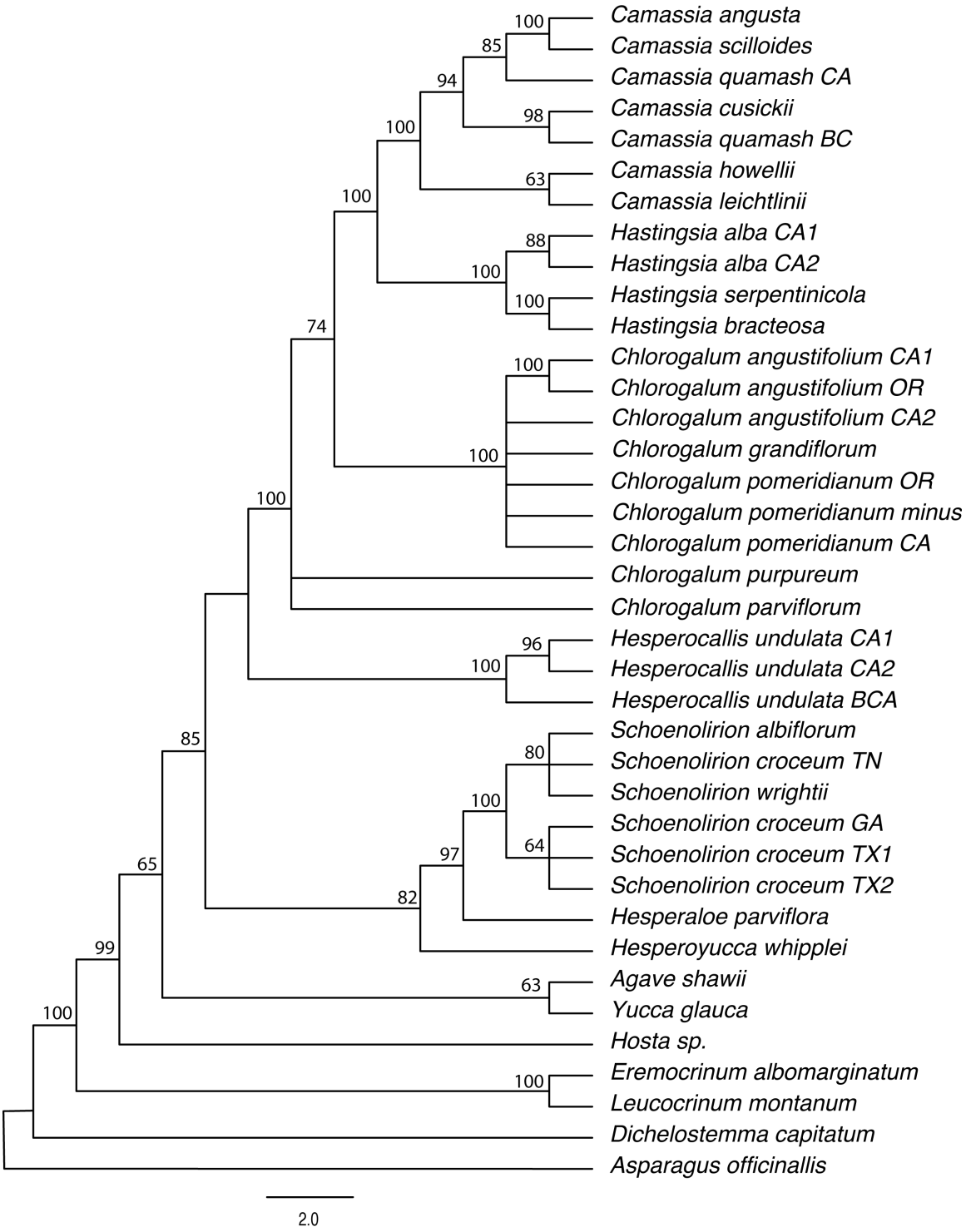

Figure 3. The maximum likelihood tree discovered by RAxML with the RAxML BS, GARLI BS, and Bayesian posterior probabilities greater than 50% presented above branches.

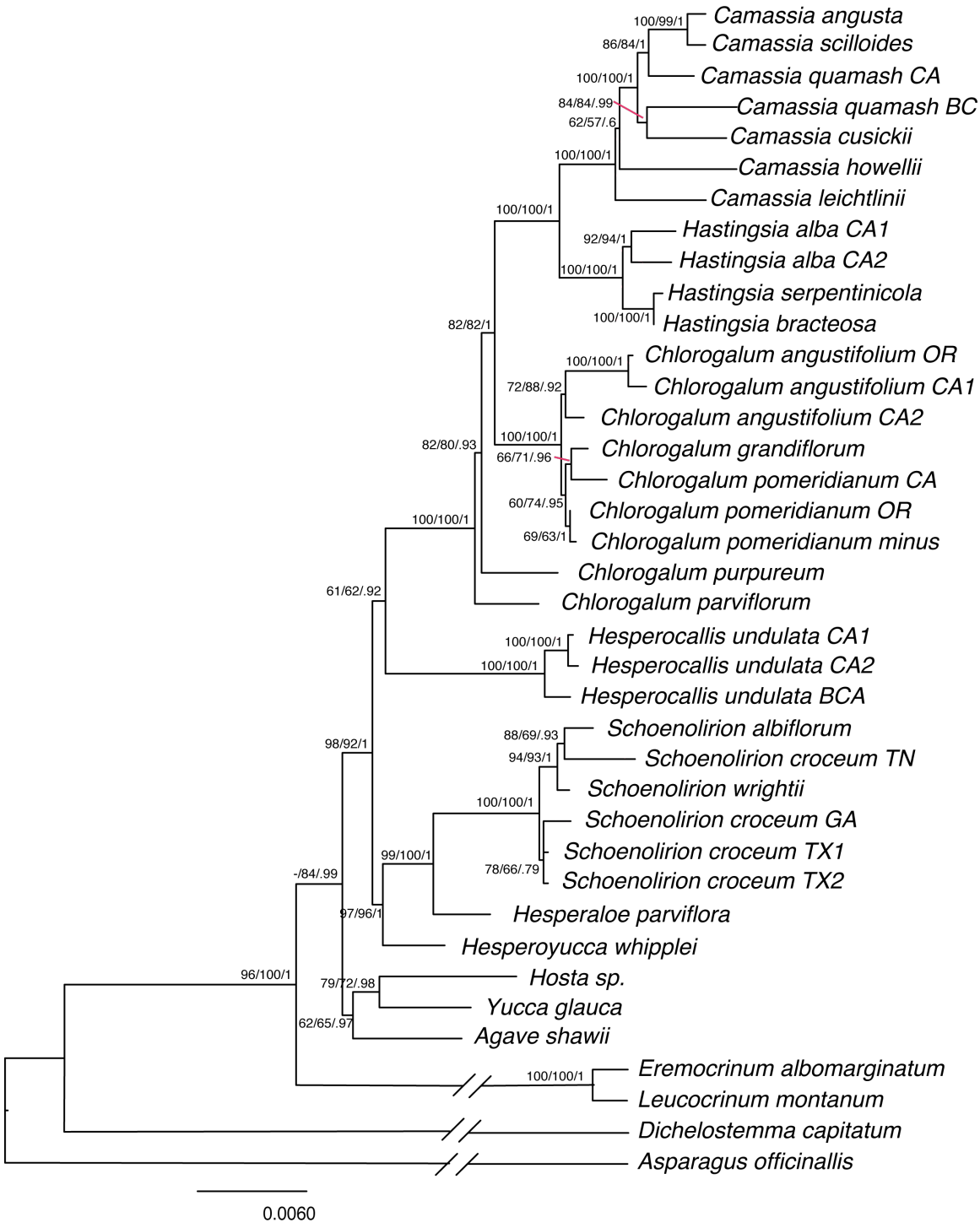

Table 6. Results of parsimony based (Templeton and Winning-Sites) and likelihood (SH) topology tests. Hypotheses 1-8 list the groups that were constrained to be (or not be) monophyletic. Groups with multiple genera were constrained as a single clade, without constraining the monophyly of the individual genera. Positive constraints of monophyly are indicated in bold, negative constraints of monophyly (the non-monophyly was constrained) are in regular font. WI and NI indicate parsimony based tests using the "with indels" and "no indels" data sets. All SH tests were performed on trees estimated form the NI data set. * = P < 0.05.

Constraint searches testing the monophyly of:	**Templeton Test: Test statistic/P-value**	**Winning-Sites Test: Test statistic/P-value**	**SH Test : Difference in -ln L/P-value**
1a. Chlorogaloideae *sensu* Speta: *Hastingsia, Camassia, Chlorogalum,* and *Schoenolirion*	**WI: 65/0.0016*** **NI: 19.5/0.0455***	**WI: 0.846 /0.0005*** **NI: 0.846/0.0225***	**39.428/0.0008***
1b. Chlorogaloideae sensu Speta + *Hesperocallis*	**WI: 0/<0.0001*** **NI: 30/0.1088**	**WI: 1.0/<0.0001*** **NI: 0.714/0.1796**	**37.239/0.007***
1c. Chlorogaloideae this study: *Hastingsia, Camassia, Chlorogalum,* and *Hesperocallis*	WI: 0/0.0016* **NI: 0/0.08333**	WI: 1.0/0.002* **NI: 1.0/0.25**	2.699/0.348
2. *Hastingsia*	WI: 0/0.0003* NI: 0/0.0003*	WI: 1.0/0.0002* NI: 1.0/0.0002*	52.346/0.004*
3. *Camassia*	WI: 0/<0.0001* NI: 0/0.0005*	WI: 1.0/<0.0001* NI: 1.0/0.0005*	48.260/0.003*
4. *Chlorogalum*	WI: 2.5/0.3173 NI: 3/0.1797	WI: 0.750/0.6250 NI: 0.800/0.3750	9.531/0.112
5. *Schoenolirion*	WI: 0/<0.0001* NI: 0/0.0009*	WI: 1.0/<0.0001* NI: 1.0/0.0010*	88.186/<0.0001*
6. *Hastingsia* and *Camassia*	WI: 7/0.0023* NI: 0/0.0002*	WI: 0.923/0.0034* NI: 1.0/0.0001*	53.570/0.003*
7. *Hastingsia, Camassia,* and *Chlorogalum*	WI: 27/<0.0001* NI: 0/<0.0001*	WI: 0.923/<0.0001* NI: 1.0/<0.0001*	74.931/<0.0001*
8. *Schoenolirion, Hesperaloe* and *Hesperoyucca*	WI: 0/0.1573 NI: 6/0.6547	WI: 1.0/0.5000 NI: 0.600/1.000	5.859/0.144

Figure 4. Parsimony ancestral state reconstruction of serpentine soil adaptation. Ancestral states in the set of most parsimonious reconstructions are indicated by black: serpentine tolerant, white: not serpentine tolerant; black/white: equivocal.

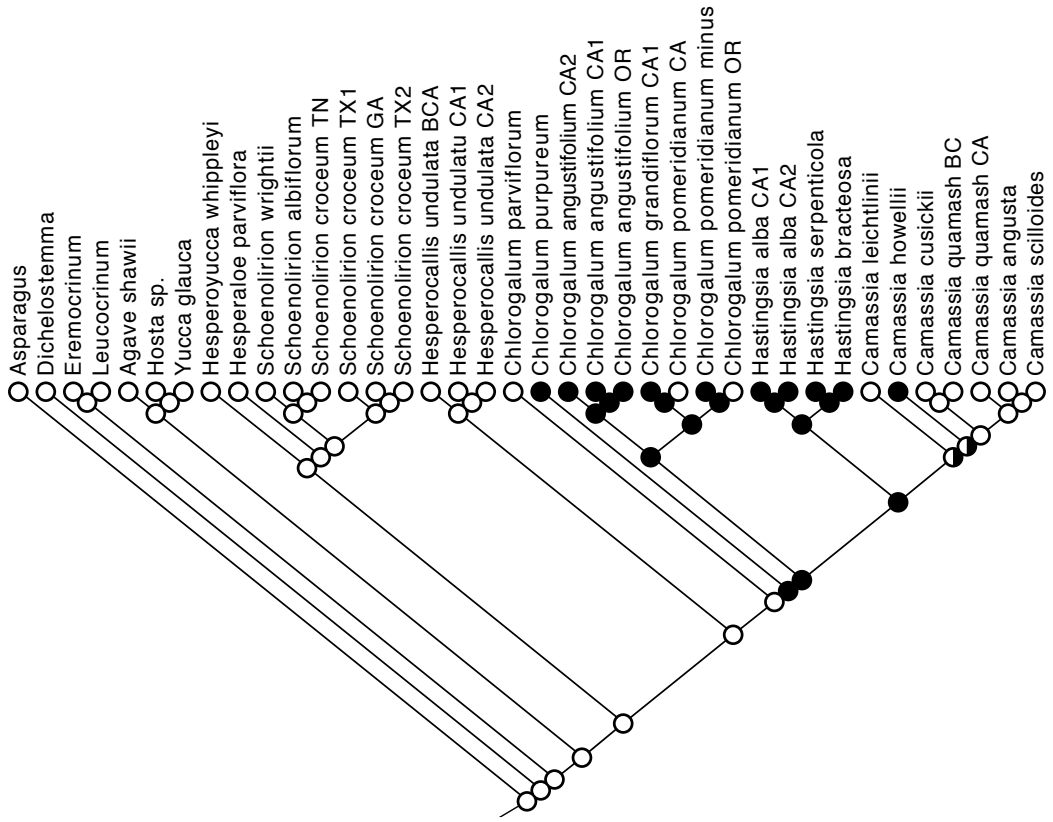

Figure 5. Likelihood based ancestral state reconstruction of serpentine soil adaptation under the Mk model. The likelihood of serpentine tolerance is indicated at each node by a pie graph, where the black area is equal to the probability of serpentine tolerance. Likelihood and posterior probabilities for numbered nodes are listed in Table X.

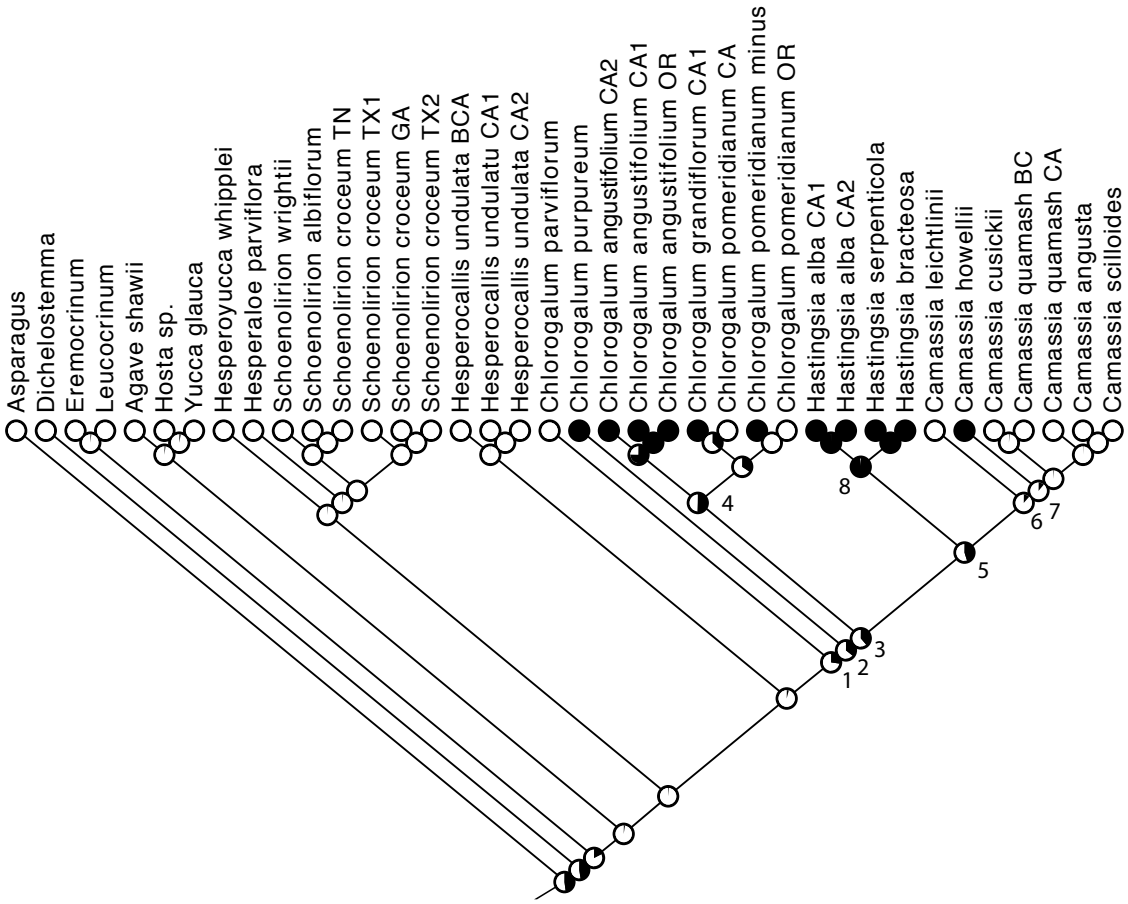

Table 7. Likelihood and posterior probabilities from the likelihood and SM ancestral character state reconstructions for serpentine tolerance in nodes 1-8 of the cladogram indicated in Figure 5.

Node	1	2	3	4	5	6	7	8
Likelihood	0.27	0.35	0.38	0.50	0.45	0.11	0.01	0.98
Posterior Probability	0.373	0.999	0.996	0.996	0.928	0.015	0.003	1.000

CHAPTER III

A CHLOROPLAST PHYLOGENY OF HASTINGSIA

Introduction

Serpentine soils have been of great interest to ecologists and evolutionary biologists because they contain toxic concentrations of metals and low essential nutrients, giving rise to plant populations with high levels of endemism (Kruckeberg 1984; Brady et al. 2005; Alexander 2007). Serpentine soils are formed by the weathering of ultramafic rocks, which are characterized by low Ca^{2+}/Mg^{2+} ratios and high levels of heavy metals such as chromium, copper, lead, and nickel. Also, the patchy nature of exposed serpentine rock creates many small and isolated populations, leading to extreme rarity in many cases. A high density of serpentine outcrops are found in southwest Oregon and northern California. Within California, serpentine endemics represent 13% of all rare, threatened and endangered taxa (Kruckeberg 1984; Safford et al. 2005), while serpentine outcrops represent a mere 1.5% of California's total land area (Harrison et al. 2000), making serpentine outcrops the site of incredible diversity. Fragmented populations, such as those inhabiting serpentine soils, are of special conservation concern due to the detrimental effects of genetic drift, inbreeding, and higher probability of extinction (Ellstrand and Elam, 1993; Young et al. 2001). Because of this, fragmented populations are more sensitive to human disturbances than more widespread populations. However, fragmentation may also be the impetus for speciation if isolated populations become genetically divergent and adapt to local

conditions (Orr and Smith, 1998). Serpentine endemics provide an important model for studying how fragmented species and populations evolve and may provide important insights into recently fragmented populations due to human disturbance. *Hastingsia* is a genus of Agavaceae containing rare species that grow on serpentine substrates. It consists of two to four species that are typically restricted to isolated outcrops of serpentine rock within the Klamath-Siskiyou region of Oregon and California, with one species ranging south to the northernmost Sierra Nevada. The closest relatives of *Hastingsia* are *Camassia* (continental U.S.) and *Chlorogalum* (SW Oregon and California) (see Chapter II). The first documented collection of *Hastingsia* was described as *Schoenolirion album,* a new species within an established genus. Watson (1879) proposed that *S. album* be segregated as the type of a new genus, *Hastingsia.* Although generally well accepted, *Hastingsia* and *Schoenolirion* continued to be considered congeneric in some treatments (Krause 1930; Hutchinson 1959). In 1991, Sherman and Becking (1991) published a study outlining the major differences between the two genera and concluded that the number of differences was significant enough to warrant the segregation of the genera, resulting in *Hastingsia* being recognized as a distinct genus in all major treatments. Furthermore, *Schoenolirion* has been found to be more closely related to *Hesperoyucca* and *Hesperaloe,* than to *Hastingsia, Camassia,* or *Chlorogalum* (Chapter II).

Until the 1980's, only two species of *Hastingsia* were recognized: *H. alba* and *H. bracteosa.* Since then, two additional species have been described by Becking (1986; 1989) and are recognized in *Flora of North America* (Becking 2002): *H. serpentinicola* is a segregate from *H. alba,* and *H. atropurpurea* is a segregate from *H. bracteosa.* Recognition of these segregates has been questioned due to difficulty in consistently observing the diagnostic differences between the species, most of which pertain to the size of leaves and inflorescences and to the color of the perianth.

According to Becking (1989), the distributions of *H. alba* and *H. serpentinicola* are sympatric throughout the Klamath-Siskiyou ranges of southwestern Oregon and northwestern

California, but only *H. alba* occurs in the northernmost Sierra Nevada and the southernmost Cascades. Becking distinguished *H. serpentinicola* as smaller than *H. alba,* occurring mainly on hillsides of serpentine rock that are wet in the spring, but drying in early summer. In contrast, he considers *H. alba* to be much more "robust", occurring in or near permanently wet bogs, but not restricted to serpentine. In Becking's keys to the genus (Sherman and Becking 1991; Becking 1993), characters used to distinguish *H. alba* from *H. serpentinicola* include overlapping values for stem and leaf length, the degree of reflexion in the perianth parts, and the habitat it occurs in (i.e., whether it occurs where water is available all year). Individuals with intermediate characters in morphology and/or habitat are not uncommon, making it difficult to distinguish between the two species.

Hastingsia bracteosa and *H. atropurpurea* are sympatric with *H. alba* and *H. serpentinicola,* but are very narrowly restricted to continually wet *Darlingtonia californica* bogs of the Illinois River Valley of southwest Oregon (Becking, 1986). Becking separated *H. atropurpurea* by its deep reddish-purple tepals and more glaucous leaves, compared to white tepals and greenish leaves in *H. bracteosa.* For the most part, their geographic distributions do not overlap, but several populations that contain plants identifiable to both species, as well as putative hybrids with intermediately colored tepals (i.e., pink), have been documented (Lang and Zika, 1997). Becking also compared morphological characters including dimensions of the bulbs, leaves, scapes, floral and inflorescence bracts, and raceme branches, the average measurements of which he found to be significantly different between the two species. However, Lang and Zika were unable to consistently distinguish these species based on these characters alone. Some species in related genera are easily distinguished by flower color, such as *Chlorogalum purpureum* and *C. parviflorum,* but are also accompanied by differences in vegetative traits (Hoover, 1940). Lang and Zika were unconvinced that differences other than perianth color segregated *H. atropurpurea* from *H. bracteosa,* and they proposed that the taxonomic rank of *H. atropurpurea* be lowered to variety, i.e., *H. bracteosa* var. *atropurpurea*.

Hastingsia bracteosa and *H. atropurpurea* are considered by the Oregon Natural Heritage Information Center (2004) to be threatened with extinction. However, recognition of *H. atropurpurea* as a distinct species is not universally accepted and resolution of its status is essential for formulating conservation plans for both species. Molecular phylogenetics can provide support for species circumscriptions, as well as reveal significant taxonomic units that harbor genetic diversity, making them higher priorities for conservation. Fragmented serpentine populations are of special conservation concern because they are more sensitive to human disturbances than more widespread populations (Ellstrand and Elam, 1993). An understanding of the evolution and relationships in *Hastingsia* will be insightful for many other rare species that occur in these fragmented and unique habitats, as well as recently fragmented habitats due to human disturbance. Furthermore, an understanding of geographical patterns and the distributions of genealogies in populations can give insights into how past demographic processes have contributed to current distributions (Avise, 2000).

By constructing a chloroplast phylogeny of *Hastingsia* with population level sampling of all four of its putative species, I aim to assess relationships among evolutionary lineages within *Hastingsia,* evaluate current species circumscriptions, and determine population history within and among species. In addition to the scientific understanding gained by this project, the work will be beneficial for conservation. Knowledge of the phylogenetic history of *Hastingsia* will add to our understanding how fragmented populations evolve, and will potentially benefit other populations undergoing fragmentation due to human disturbance. Furthermore, the characterization of inter- and intraspecific relationships within *Hastingsia* will inform conservation efforts related to rare and narrowly endemic varieties.

Methods

Taxon Sampling— A total of 47 individuals from 43 populations of *Hastingsia* were collected, including all four putative species across their respective geographic distributions

(Table 7). Hereafter, accessions will be referred to by their taxon and population number, for example, *H. alba 08* is found in population 8 located on the map (Fig. 6). The four accessions from population 17 of the Illinois Valley, OR, were identified as putative hybrids between *H. serpentinicola, H. bracteosa,* and *H. atropurpurea,* and accessions from populations 13, 14, 15, and 32 were unidentifiable to species level because they lacked inflorescences. Remaining individuals were identified to species using the taxonomic keys in the Jepson Manual (Hickman 1993). The outgroup taxa consisted of representative species from each genus of Chlorogaloideae, *Chlorogalum parviflorum, C. purpureum, C. angustifolium, C. pomeridianum*, *Schoenolirion croceum, Camassia leichtlinii, C. quamash,* and *C. howellii.* Also included in the outgroup are other members of Agavaceae (*Hesperocallis undulata, Hesperoyucca whipplei,* and *Hesperaloe parviflora*), which have been found to be closely associated with members of Chlorogaloideae (Bogler and Simpson 1996; Fay and Chase 1996; Smith et al. 2008).

Character sampling—Genomic DNA was extracted from silica-dried leaf material collected in the field (in the case of all *Hastingsia* specimens) or from herbarium specimens using a commercial kit (Wizard® Genomic DNA Purification Kit, Promega, Madison, Wisconsin, DNeasy® Plant Mini Kit, Qiagen, Valencia, California). Polymerase Chain Reaction (PCR) was used to generate templates for sequencing. Three plastid regions, the *rpl16* intron, and the *trnD–trnY–trnE–trnT* and *psbJ-petA* intergenic spacers, were amplified using "universal" primers or primers newly developed for this study (Table 3), when nonspecific amplification occurred or if genomic DNA was severely degraded in the case of some herbarium material. Reactions were carried out with the iCycler® or C1000® (Bio-Rad Laboratories, Hercules, California) thermal cyclers. Amplicons were generated using a standard 50 or 25 µl reaction consisting of 1 µl of genomic DNA undiluted or diluted by a factor of 10–50, 0.5 mM of both forward and reverse amplification primers, 200 µM dNTPs, 1.5 mM $MgCl_2$, 1× reaction buffer supplied by the polymerase manufacturer, 5% DMSO, and 0.2 U *Taq* DNA polymerase (Promega, Madison, Wisconsin). Difficult templates were amplified in similar reactions, for which HotMaster® *Taq*

DNA polymerase (Eppendorf, Westbury, New York) and supplied buffer were substituted and DMSO was omitted. PCR reactions were conducted with the following cycling conditions: 30 or 35 cycles of 95°C for 1 min, 51°C for 1 min, 65°C for 4 min, followed by a final extension at 65°C for 8 min and a final hold at 4°C. Amplicons were purified for DNA sequencing by column filtration (Wizard® SVGel and PCR Cleanup System, Promega, Madison, Wisconsin). DNA sequences were obtained by direct cycle sequencing with ABI Prism® BigDye® Terminator v3.1 (at Portland State University) or v1.1 (at Oklahoma State University) Cycle Sequencing Kit (Applied Biosystems, Foster City, California) following the manufacturer's protocol. Unincorporated dye terminators were removed by centrifugation through columns of Sephadex™ G-50 Fine (GE Healthcare Bio-Sciences, Piscataway, New Jersey) or by ethanol/EDTA/sodium acetate precipitation following the BigDye manufacturer's protocols. Dye-labeled fragments were visualized and analyzed on the ABI Prism® 3100 Genetic Analyzer (Applied Biosystems,) at the Oregon Health and Science University Sequencing Core or on the ABI 3730 DNA Analyzer (Applied Biosystems) at the Oklahoma State University Recombinant DNA and Protein Core Facility. Sequencing primers were selected to give complete double stranded coverage of each region to maximize accuracy and included both "universal" primers and new primers developed from an alignment of *Camassia*, *Hastingsia*, *Chlorogalum*, *Schoenolirion,* and *Leucocrinum* sequences (Table 3). Complete sequences were assembled and edited with the SeqMan™II module of Lasergene ver. 6 (DNASTAR, Madison, Wisconsin).

Phylogenetic Analyses— Sequences were aligned by eye for each region with Se-Al ver. 2.0 (Rambaut 1996). All alignment positions containing ambiguously aligned regions were omitted from phylogenetic analyses. Two inversions, one from the *trnD-T* locus (Inv1) and one from the *rpl16* intron (Inv2), were coded as binary characters in the alignment, but were each analyzed with the concatenated sequence data separately.

Phylogenetic trees were inferred under the maximum parsimony (MP) and maximum likelihood (ML) criteria and by Bayesian inference (BI). All analyses were conducted on the

concatenated sequences from the three different chloroplast gene regions with the assumption that recombination between the loci is unlikely to occur in the plastid chromosome. Maximum parsimony trees (MPTs) were sought using two different approaches. In the first approach, heuristic searches were implemented with PAUP* ver. 4.0b10 (Swofford 2002) and performed with 1000 replicates of stepwise random addition of sequences, holding 1 tree per step, followed by tree-bisection-reconnection (TBR), keeping a maximum of 100 trees of scores greater than or equal to one per replicate with 'MaxTrees' set to 10^7. Second, ratchet analyses (Nixon 1999) were implemented with PAUPRat v1 (Sikes and Lewis 2001) and PAUP* via the CIPRES Science Gateway v3.1 (www.phylo.org). Twenty separate ratchet analyses were conducted with 200 rounds each. For each round, 20% of the informative characters were reweighted, followed by a heuristic search, with a single replicate of stepwise random addition of sequences, keeping one tree subject to TBR branch swapping. MPTs found in all 20 analyses were summarized with a single strict consensus tree. Topology, tree scores, and strict consensus trees were compared from the sets of MPTs generated in the heuristic search and the ratchet search in order to evaluate the thoroughness of the parsimony tree searching methods.

Clade support for the parsimony trees was assessed using nonparametric bootstrapping (BS) (Felsenstein 1985) implemented in PAUP* with 5000 pseudoreplicates and ten random-addition-starting sequences with trees subjected to TBR branch swapping , keeping a maximum of 20 trees greater than or equal to a tree score of one for each pseudoreplicate.

The two coded inversions, *Inv1* and *Inv2,* were each analyzed with the concatenated dataset by performing 20 separate ratchet analyses each and by estimating clade support by bootstrapping using the identical methods listed above.

ML trees were estimated using the programs RAxML v7.0.4 (Stamatakis 2006) and GARLI v0.96 (Zwickl 2006). Tree estimation using RAxML was performed with the concatenated sequences partitioned by locus, with the GTR+Γ (GTRGAMMA) model of nucleotide substitution assigned to each partition, but with parameter estimates free to vary across

partitions. Support values were obtained with RAxML by conducting 5000 pseudoreplicates utilizing the rapid bootstrapping algorithm (with the default of 25 rate categories). Tree estimation using GARLI was implemented without data partitioning (not an available option), and with the GTR+ Γ model of nucleotide substitution. Clade support using GARLI was obtained by conducting 100 (the maximum permitted) bootstrap replicates. Both RAxML and GARLI analyses were implemented via the online CIPRES Portal..

BI was conducted using MrBayes, v3.12 (Ronquist and Huelsenbeck 2003). The optimal evolutionary model of nucleotide substitution for each cpDNA locus was chosen by applying hierarchical likelihood ratio tests (hLRTs) and the Akaike Information Criterion (AIC) as implemented in MrModeltest, v2.3 (Nylander 2004). Independent substitution models for each of the cpDNA loci were employed by creating partitions in the concatenated data set. Metropolis-coupled Markov chain Monte Carlo simulations were run with eight linked chains (seven heated and one cold) and default priors for all model parameters, except for the parameter controlling the temperatures of heated chains, which was reduced to 0.02. Two independent runs of 1×10^7 generations were compared to assess convergence to a stationary distribution of parameter samples by examining the average of standard deviations between the two runs of split frequencies in MrBayes and by examining the effective sample size (ESS) for each parameter using Tracer, version 1.4 (Rambout and Drummond 2009). A cut-off of 0.01 standard deviations and ESS's greater than 200 were used as guidelines to assess convergence of runs. After a burnin of 2.5×10^6 generations, parameter values (including trees) were sampled every 1000 generations from the stationary distribution to calculate posterior probabilities of parameters.

Unrooted, statistical parsimony networks were generated using the program TCS v1.21 (Clement et al. 2000). Because of potential problems with missing or ambiguous data while assigning sequences to haplotypes in TCS, sequences with large deletions and/or missing data, as well as characters with missing data (including gaps created by differences in the length of mononucleotide repeats), were removed from the dataset. Remaining gaps were treated as

missing data. Three separate analyses were conducted with connection limits set to 24, 10, and 8 steps between haplotypes.

Hypothesis testing—The Templeton (Wilcoxon signed-ranks;(1983), Winning-sites (Prager and Wilson 1988), and Shimodaira– Hasegawa (SH; (Shimodaira and Hasegawa 1999; Goldman et al. 2000) tests (implemented in PAUP*) were used to evaluate whether constraining taxonomic groups to be monophyletic resulted in significantly different topologies from the trees estimated in the unconstrained search. The monophyly of each of the four species was tested, as well as the monophyly of the species pairs, *H. alba/serpentinicola,* and *H. bracteosa/atropurpurea*. The monophyly of *H. alba* was tested in two ways: 1a includes all accessions identified as *H. alba* and 1b excludes individuals from populations 25 and 33 of the Illinois Valley, OR, because they were recovered in the clade containing all *H. serpentinicola, bracteosa,* and *atropurpurea* accessions. A summary of the six monophyletic constraints and test results are presented in Table 10. A total of eight accessions were omitted from all hypothesis tests, including all four putative hybrids from population 17 and unidentified individuals from populations 13, 14, and 15. For the parsimony-based Templeton and Winning-sites tests, heuristic searches (run under parameters used for the original unconstrained heuristic search) were performed with and without constraints, comparing the strict consensus trees from each analysis. For the likelihood based SH test, I used GARLI to estimate the ML trees with and without constraints. The SH-test was performed with the GTR + Γ model of nucleotide substitution to best match the model implemented in GARLI, using the RELL method and performing 1000 bootstrap pseudoreplicates.

Results

Sequence characteristics— The alignment for all three loci was easily accomplished by eye, although numerous short gaps were introduced. Within the *psbJ-petA* spacer, a 577 bp deletion was discovered in *H. sp. 32.* Very low variation was found among the *Hastingsia*

sequences, with a total of 37 parsimony informative sites found across the three loci, compared to 117 found with outgroup taxa included. A summary of sequence characteristics is presented in Table 8. Two inversions (*Inv1* of *trnD-T* and *Inv2* of *rpl16*) were found to be variable between *Hastingsia* and outgroup sequences. These were analyzed with the concatenated sequences in separate data sets. However, their inclusion did not change the topology of the tree nor did it significantly change clade support values. No indels were found to be variable among *Hastingsia* sequences.

Model selection— Best fitting models of evolution differed across the regions. For the *trnD-T* spacers, alternative model selection procedures preferred different models. Paths through the tree of hLRTs evaluated by MrModeltest resulted in the HKY + Γ, GTR + Γ, and GTR +I models, however, the AIC implementation of MrModeltest selected the GTR + Γ model, which was used in the Bayesian analysis because it was preferred in both methods. For the *rpl16* intron, hLRTs preferred either the HKY + Γ or HKY + I, and AIC preferred HKY + Γ, which was selected for use. For the *psbJ-petA* spacer, all hLRTs selected either the GTR + I or GTR + Γ models while the AIC preferred GTR + Γ, which was used in the BI analysis. Table 9 presents the ML parameter estimates and Bayesian credible intervals for substitution models selected for use.

Tree searches and support— MP, ML, and BI analyses produced phylogenetic estimates that were largely congruent; however, some relationships differ in resolution or conflict among tree estimation procedures. MP analyses for the conventional heuristic and the ratchet searches produced consensus trees identical in both length and topology (Figure 7). The ML trees estimated in RAxML and GARLI and the 50% majority rule consensus of trees sampled from the stationary phase of the BI analysis were identical in topology, which is represented by the RAxML tree (Figure 8) with bootstrap percentages (BS) and posterior probabilities (PP) for nodes presented above their subtending branches.

Hastingsia was highly supported as monophyletic in all analyses (MP BS = 100, RAxML BS = 100, GARLI BS = 100, PP = 1), however no species of *Hastingsia* were found to be monophyletic. Three major clades were recovered in all analyses, and *H alba* accessions were placed in each major clade. Clade A (86, 90, 86, 1) is composed of all accessions of *H. serpentinicola, H. bracteosa,* and *H. atropurpurea,* and also includes two *H. alba* accessions. Within it, there is little resolution and the few recovered clades are weakly supported. At the very base of this clade is the unidentified *H. sp. 15*, found as sister to a clade containing the remaining individuals (57, 55, 54, .82). There are three weakly supported subclades within Clade A, and these with the remaining individuals form a large polytomy. The second major clade (Clade B; 89, 96, 90, 1) contains only *H. alba* individuals and two of the four unidentified individuals (*H. sp. 13, 14*). Clade B is moderately supported (69, 79, 56, .96) as sister to Clade A. Lastly, Clade C is sister to all other *Hastingsia* accessions, and although clade support is low (60, 67, 57, .88), it was recovered in every analysis. This clade consists of *H. alba* accessions only, with a well supported clade of individuals from the Sierra Nevada (SN clade; 93, 97, 91, 1). The remaining individuals in Clade C are found in the Trinity Mountains (*H. alba* 1, 2, 9, 10), except for *H. alba 34,* which is found further north in the southern Siskiyou Range.

Hypothesis testing— Results of the tests of taxonomic hypotheses are presented in Table 10. Hypothesis 1 tested the monophyly of *H. alba,* with 1a including all accessions identified as *H. alba,* and 1b omitting *H. alba 25* and *33* that were found in the clade with all *H. serpentinicola, H. bracteosa,* and *H. atropurpurea* accessions. Trees constrained to force all *H. alba* accessions to be monophyletic resulted in significantly less optimal trees than the unconstrained trees when *H. alba* 25 and 33 were included (Hypothesis 1a) for the Templeton and SH tests, but not the WS test. When *H. alba* 25 and 33 were excluded (Hypothesis 1b), the optimal topologies of the constrained search were not found to be significantly less optimal than the unconstrained search in any test. Likewise, trees constrained to recover individual monophyletic groups of *H. serpentinicola* (Hypothesis 2), *H. bracteosa* (Hypothesis 3), and *H.*

serpentinicola (Hypothesis 4) did not differ significantly from the unconstrained topologies in any test, with the constrained tree for the monophyly of *H. atropurpurea* having a difference of 0 between constrained and unconstrained trees in both the MP and ML analyses. Constraining *H. alba* and *H. serpentinicola* to be collectively monophyletic without constraining the monophyly of each species (Hypothesis 5), did not recover trees that were significantly longer in parsimony based tests, but the constrained ML tree was significantly less likely in the SH test. Lastly, constraining *H. bracteosa* and *H. atropurpurea* to be collectively monophyletic, also without constraining the monophyly of the individual taxa, resulted in trees that were not significantly worse in either the MP or ML analyses.

Haplotype network— Among the 44 sequences obtained, 24 different haplotypes were observed, with one haplotype represented by 13 individuals, one represented by four individuals, and three represented by two individuals. A parsimony connection limit of 24 (95% connection limit) resulted in a single network (not shown). Reducing the number of steps between haplotypes to ten (99% connection limit) produced two networks (not shown), and further reducing the number of steps to eight resulted in four networks, which is presented in Figure 9. A limited connection limit of eight steps was preferred in order to minimize the number of potentially homoplasious substitutions between inferred or unsampled haplotypes. Network A consists of 23 individuals found within the Illinois Valley and surrounding areas, with the ancestral haplotype inferred to be that of *H. serpentinicola 16*, which is shared by 12 other individuals. Networks B and C are both composed of individuals found throughout the eastern Siskiyou Mountains and Klamath subranges. Network SN consists of all seven individuals from the Sierra Nevada. *Hastingsia alba* is placed in all four networks, however all *H. serpentinicola, H. bracteosa,* and *H. atropurpurea* accessions are placed in network A, with all *H. atropurpurea* accessions sharing the ancestral haplotype.

Discussion

No species of *Hastingsia* were found to be monophyletic in any of the tree estimation methods. All individuals of *H. serpentinicola, H. bracteosa,* and *H. atropurpurea*, plus two *H. alba* were placed in a large polytomy (Clade A), and the remaining *H. alba* individuals forming two clades paraphyletic to Clade A. However, statistical topology tests neither refute nor support the lack of monophyly with one exception. When all accessions of *H. alba* were constrained to be monophyletic, the topologies were found to be significantly worse in the Templeton and SH tests, but not the WS test. When *H. alba* 25 and 33 (the two accessions found in Clade A with *H. serpentinicola, H. bracteosa,* and *H. atropurpurea*) were not constrained to be monophyletic with the rest of *H. alba,* the constrained searches did not result in significantly different topologies in any test, thus these two "outliers" were responsible for the significance of *H. alba* non-monophyly. I also tested the monophyly of all *H. alba* and *H. serpentinicola* accessions, without constraining the individual species monophyly, to test the hypothesis that *H. serpentinicola* is derived from *H. alba* based on their morphological similarities. However, only the SH test indicated that the constrained topology was significantly worse, providing a weak refutation of this hypothesis. Similarly, constraining *H. bracteosa* and *H. atropurpurea* to be one monophyletic lineage, without constraining the monophyly of the individual species, did not result in significantly worse topologies.

If adhering strictly to a biological (Wright 1940; Mayr 1942; Dobzhansky 1950) or phylogenetic (Donoghue 1985; Mishler 1985; Baum and Shaw 1995) species concept, the four species in *Hastingsia* would not be considered distinct. Evidence of hybridization (Lang and Zika 1997; personal observations) would refute the biological species concept because these groups are able to interbreed, indicating a lack of reproductive isolation. However, hybridization appears to be minimal and limited to only the narrow zones of contact between species. Similarly, the phylogenetic species concept requires that only monophyletic lineages be considered species, which would refute species distinction in at least *H. alba,* but the data only

indicate a lack of evidence for the monophyly of the remaining species. However, under a unified species concept described by de Queiroz (2007), recently diverged taxa are considered to be independently evolving lineages that are not diagnosable by a single criterion such as allelic monophyly or reproductive isolation. Under the unified species concept, species can recognized even in the presence of incomplete reproductive barriers and incomplete lineage sorting.

Hybridization and subsequent introgression is one likely explanation for species non-monophyly in *Hastingsia*. The patterns of morphological variation are consistent with the presence of four distinct taxa, in which case the population-level chloroplast phylogeny reflects gene flow due to incomplete barriers to reproduction after secondary contact. Indeed, there is evidence for hybridization among the taxa. Lang and Zika (1997) observed putative hybrid intermediates between *H. bracteosa* and *H. atropurpurea*. Tepal color in the supposed hybrids ranged in color from white to pink to purple. Lang et al. (1994) observed that presumed hybrid intermediates between *H. bracteosa* and *H. atropurpurea* had no observable differences in pollen viability or seed set compared to non-hybrid individuals, and found no fixed isozymic allele differences between populations of the two species. They concluded that *H. atropurpurea* did not merit species status. However, they did not consider that the lack of isozymic allele fixation may be due to an insufficient time for recently diverged populations to show fixation, rather than ongoing gene flow. *Hastingsia bracteosa* and *H. atropurpurea* maintain non-overlapping distributions for the most part. Only in the few zones of sympatry are intermediate, putatively hybrid individuals found. Therefore, it would seem that hybridization is limited and other processes are responsible for the observed lack of variation among sequences. Similarly, I observed a mixed population (17 from the Illinois Valley, Oregon; see Fig. 6) containing individuals that I identified as *H. serpentinicola, H. bracteosa,* and *H. atropurpurea* growing in close proximity and with individuals morphologically intermediate to all pairs of the three taxa. I have made additional casual observations of putative hybrids between *H. serpentinicola* and *H. bracteosa* or *H. atropurpurea* elsewhere. However, such hybrid individuals are highly localized

and have not led to questions concerning the specific distinction between *H. alba s.l* and *H. bracteosa s.l.* Thus, introgression may in part cause patterns of species non-monophyly in the chloroplast phylogeny, but because hybridization seems to be limited to a few narrow zones of contact, it may not be the sole cause.

Species non-monophyly is commonly observed in recently diverged taxa, even in the absence of introgression (Maddison 1997). If species of *Hastingsia* have either recently and/or rapidly diverged, then a plausible explanation for species non-monophyly in the chloroplast phylogeny may be the presence of incomplete lineage sorting (ILS), which occurs when multiple alleles present in an ancestral population fail to become fixed prior to speciation. When populations diverge during speciation, the alleles may randomly sort into the daughter populations. Consequently, the phylogeny of the locus may not match the true phylogeny of the populations because there may be allelic variation that arose before population divergence, but randomly sorted into the new populations. The probability of discordance between the relationship of alleles and the relationships of their species increases as effective population size increases and time between speciation events decreases (Maddison 1997). In order to observe complete lineage sorting (a gene phylogeny that is congruent with the species phylogeny), the alleles must become fixed before the daughter populations diverge, with the time to fixation dependent on effective population size. Therefore, in rapidly and/or recently diverged lineages, allelic fixation may not have occurred prior to speciation. The low levels of resolution, especially within Clade A, may be evidence for rapid diversification in the chloroplast phylogeny, which would create conditions favorable to incomplete lineage sorting. However, disentangling the effects of introgression from ILS would require more intensive sampling of individuals and data from unlinked loci (Carstens and Knowles 2007; Liu and Pearl 2007; Meng and Kubatko 2009).

Another explanation for lack of species monophyly in *Hastingsia* is that there is not sufficient variation in the data with only a few loci sampled to resolve relationships. The influence of this factor is suggested by the prevalence of non-significant hypothesis tests of

species non-monophyly. Observed morphological variation may indicate the boundaries of distinct evolutionary lineages, however in recently diverged populations or species, observing a direct relationship between morphological and genetic variation is less likely when examining slowly evolving genomic regions. Chloroplast sequences are not particularly fast evolving. Many other phylogenies at the species level based solely on chloroplast sequences are unresolved. In a phylogeny of *Calochortus* (Liliaceae) using three chloroplast loci (*trnT-trnF, psbA-trnH,* and *rpl16*), Patterson and Givnish (2004) found little resolution. In some cases, multiple species were recovered in a single polytomy. Similarly, a three-locus cpDNA phylogeny of *Lepidium* (Brassicaceae; Mummenhoff et al. 2001) found low or no resolution within and among species. The fixation of different sets of alleles and the accumulation of novel alleles in separate populations will leave genetic signatures, but how quickly this is detectable will depend on the molecular marker as mutation rates are highly variable. Therefore, chloroplast loci may lack a detectable phylogenetic signal between recently diverged lineages, or between lineages that rapidly diverged long ago. Low resolution within the phylogeny could explain the lack of structure and therefore the lack of monophyly for *H. bracteosa, H. atropurpurea*, and *H. alba/serpentinicola* within Clade A. Thus, the lack of specific monophyly should be interpreted with caution as evidence for lack of species level distinction.

Lastly, the idea that there is only one morphologically variable species of *Hastingsia* is the most simple, but least plausible explanation for the patterns found in the chloroplast phylogeny. Some floristic treatments have already failed to recognize a distinction between *H. alba* and *H. serpentinicola*, such as the Oregon Flora Project (Cook and Sundberg 2011). Similarly, *H. atropurpurea* is treated as a variety of *H. bracteosa* by the Oregon Flora Project and the USDA (www.plants.usda.gov). On the other hand, specific distinction between *H. alba s.l* and *H. bracteosa s.l* has not been contested because morphological variation between the taxa is clear and consistent. If only one morphologically variable species is present, we would expect there to be no reproductive barriers, and no lineage divergence among populations. However,

hybridization seems to be minimal and confined to narrow zones of contact, indicating that at least some barriers to gene flow are present. Also, the presence of some well-supported clades within the genus is evidence for lineage divergence. Therefore, the lack of monophyly is not likely to be an indication of only a single species in the genus.

Despite the lack of species monophyly, there was significant population structure within the genus, indicated by strong support for clades and lengthy connections in the haplotype network. This structure corresponds well with geography. All individuals from the Sierra Nevada form a highly supported subclade within clade C (Figs. 7 and 8) and also comprise the distinct haplotype network SN (Fig. 9). The remaining individuals of clade C from populations 1, 2, 9,10, and 34 are all from the more southern Trinity Ranges, geographically proximal to the Sierra Nevada, except for *H. alba* 34, which is found much further north. These individuals also solely comprise haplotype network C (Fig. 9). Individuals from clade B and network C (13, 14, 27, 28, 29, and 37) are distributed throughout the more northern central Marble, Salmon, and Trinity Alps Ranges in California, except for population 37 which is found in the southern Siskiyou Range roughly five miles from population 34, and populations 13 and 14, which are found in the southeastern Siskiyou Ranges, east of the Illinois Valley in southernmost Oregon. Furthermore, all individuals from the Illinois Valley and surrounding mountains form a strongly supported clade C (Fig. 7 and 8) that forms the distinct haplotype network A (Fig. 9).

However, not all well supported relationships are explained by geography. Interestingly, populations 34 and 37 are found very close together geographically, but individuals from these populations are not recovered in the same clade. *H. alba* 34 was recovered at the very base of Clade C, while *H. alba* 37 was found in Clade B. It is possible that this relationship is evidence for the origin of *Hastingsia* in this area, since *H. alba* 34 is the earliest diverging population within Clade C. However, the basal placement of *H. alba* 34 was poorly supported. Moreover, the haplotype network infers the ancestral haplotype in network C to be that of *H. alba* 9, although this may be due to more intensive population sampling near population 9. If Clade C

originated near population 9, this would indicate a diversification for that clade in two directions, one from the Trinity Mountains east to the Sierra Nevada and another from the Trinity Mountains north to the southwestern Siskiyou ranges near population 34. In another geographically disparate clade, *H. alba* 27 and 28 were placed in a highly supported clade with *H.* sp. 13 and 14, but *H. alba* 27 and 28 are more geographically proximal to *H. alba* 29 and 30.

The apparent lack of geographic structure in some cases may be evidence for a relictual, more widespread distribution of *Hastingsia*. One way in which serpentine tolerant or endemic species are thought to arise is from a formerly widespread species that over time becomes restricted to serpentine habitats through multiple independent adaptations (depleted or paleoendemic species) (Stebbins 1942; Kruckeberg 1954; Kruckeberg 1957; Raven 1964; Stebbins and Major 1965; Kruckeberg 1984). In a study of *Streptanthus glandulosus* (Brassicaceae), a species found mostly on serpentine, Kruckeberg (1954, 1957) found evidence in support of the paleoendemic hypothesis. Kruckeberg determined that there were at least two serpentine biotypes, and interpreted this as evidence for the possible existence of many biotypes that have been reduced to mainly serpentine tolerant ones. In a cpDNA phylogenetic study of this species complex, Mayer and Soltis (1994) found evidence for multiple origins of serpentine tolerance, with non-serpentine populations most closely related to serpentine populations that were geographically proximal. This phylogeny also recovered taxa that were para- and polyphyletic. However, the nuclear ribosomal ITS phylogeny (Mayer and Soltis 1999), had several conflicting relationships with the cpDNA phylogeny, which were attributed to ILS and introgression.

Hastingsia, like *Streptanthus,* has a relatively widespread distribution and occurs both on and off of serpentine substrates, and therefore may also be a paleoendemic taxon. *H. alba*, according to Becking (1989), occurs both on and off serpentine soils. This study does not include any *Hastingsia* accessions that occur off serpentine (despite intensive effort to find such populations), but the possible presence of two biotypes in *Hastingsia*, serpentine tolerant and

intolerant, may be evidence that *Hastingsia* once had a much wider distribution both on and off serpentine and over time has become restricted to mostly serpentine habitats, making *Hastingsia* a paleoendemic taxon. This phenomenon, along with incomplete lineage sorting, may explain the current phylogeny in which not all taxa are recovered as monophyletic and some relationships are not explained by geography. As the distribution of *Hastingsia* became more restricted, multiple chloroplast genotypes within the wider populations could have become randomly sorted into the smaller ones. This process could explain why there are large-scale relationships that can be explained by geography, but some fine scale relationships lack geographic patterning.

Alternatively, the current distribution of *Hastingsia* may be due to range expansion through long distance seed dispersal rather than range contraction. *Hastingsia* may have originated as a much smaller population that came to occupy new serpentine outcrops through some mechanism of seed dispersal. However, mechanisms of dispersal for *Hastingsia* are largely unknown. The seeds are small and somewhat round, which could be dispersed short distances by some combination of wind, water, and/or gravity. It is also possible that animals browse the infructescences and disperse the seeds in feces. I have casually observed evidence of browsing in some populations of *Hastingsia*, but no formal study has been done and therefore this aspect of *Hastingsia* ecology largely unknown. The possible scenarios for the current distribution of *Hastingsia* are intriguing, and should be addressed in future studies.

Overall, none of the four species of *Hastingsia* sensu Becking (2002) were found to be monophyletic, but their non-monophyly was not statistically significant. Because of the slowly evolving nature of the chloroplast genome, there may not be sufficient variation in the data set to resolve relationships at this level, giving added reason to interpret these findings with great caution. However, large-scale geographic structure within the genus was found, with each of the three major clades recovered in the phylogeny corresponding to geographical regions. Incomplete lineage sorting along with introgression may cause the non-monophyly of species within the

genus. Additional research on *Hastingsia* is clearly needed. Future studies should include common garden and hybridization studies and phylogenetic and population genetic work with additional molecular data. Becking (1989) attempted a common garden experiment with *H. alba* and *H. serpentinicola*, and found that these two "flowered and produced viable seeds," and that the offspring "maintained their distinctive characters." However, since the distinctiveness of these characters has been questioned, this experiment should be repeated. In order to address the possibility of incomplete lineage sorting and introgression, more molecular data should include not only additional chloroplast loci, but also nuclear loci from independently evolving regions. Furthermore, the use of rapidly evolving microsatellite data may provide insight to the genetic distinctions of the putative species pairs *H. bracteosa* /*H. atropurpurea* and *H. alba/H. serpentinicola*. Microsatellite loci, for example, have been shown to be more variable than isozyme loci in many analyses (Lanzaro et al. 1995; Raybould et al. 1999; Sun et al. 2001). Lastly, an investigation into the dispersal mechanisms of *Hastingsia,* along with a more resolved phylogeny may provide information on the past and current distributions of the genus, which may in turn provide insights to mechanisms of how other plants come to occupy serpentine habitats.

Table 8. Population locations and vouchers for samples analyzed in this study.

Taxon name and population code	Location	Coordinates	Voucher
H. alba 01	Philpot creek, near Peanut, Shasta-Trinity National Forest, Trinity Co., CA.	N 40°27.849' W 123°10.880'	Halpin 3
H. alba 02	Near Wildwood, Shasta-Trinity National Forest, Trinity Co., CA.	N 40°21.682' W 123°12.798	Halpin 6
H. alba 03	Near Camp 18, west of Lake Oroville, Butte Co., CA.	N 39°37.440' W 121°11.347'	Halpin 12
H. alba 04	Serpentine Valley, near Virgilia, Plumas National Forest, Plumas Co., CA.	N 40°01.465' W 121°09.044'	Halpin 13
H. alba 05	Near Virgilia. Plumas National Forest, Plumas Co., CA.	N 40°00.781' W 121°07.591'	Halpin 14
H. alba 06	Bean Creek, near Virgilia. Plumas National Forest, Plumas Co., CA.	N 39°58.698' W 121°05.497'	Halpin 16
H. alba 07	Greenville, Plumas National Forest, Plumas Co., CA.	N 40°11.899' W 120°58.452'	Halpin 17
H. alba 08	Willow lake. Lassen National Forest, Plumas Co., CA.	N 40°24.411' W 121°21,763'	Halpin 20
H. alba 09	Reagan Meadow. Shasta-Trinity National Forest, Shasta Co., CA.	N 40° 18.221' W 123°02.661'	Halpin 7
H. alba 10	Near Tedoc Gap. Shasta-Trinity National Forest, Tehama Co., CA.	N 40°14.499' W 122°54.082'	Halpin 8
H. alba 11	Cherry Hill Meadow. Lassen National Forest, Butte Co., CA.	N 40°06.078' W121°30.068	Halpin 21
H. serpentinicola 12	Lone Mt Road, near O'Brien, Josephine Co., OR.	N 42°03.10' W 123°44.64'	Fishbein 5932
H. sp. 13	Near Dutchman's Peak, Jackson Co., OR.	N 42°02.35' W 122°53.47'	Fishbein 6010
H. sp. 14	Cow Creek, Jackson Co., OR.	N 42°00.86' W 122°53.12'	Fishbein 6022
H. sp. 15	Miller Lake, Josephine Co., OR.	N 42°03.83' W 123°18.22	Fishbein 6030
H. serpentinicola 16	Myrtle Creek, near Crescent City, CA.	N 41.80383 W 124.05906	Halpin 61
H. hybrid 17a	Days Gulch at Josephine Cr. - white tepals, , Josephine Co., OR.	N 42.22124 W 123.70628	Halpin 50
H. hybrid 17b	Days gulch at Josephine Cr. - purple tepals, Josephine Co., OR.	N 42.22124 W 123.70628	Halpin 51
H. hybrid 17c	Days gulch at Josephine Cr., Josephine Co., OR.	N 42.22124 W 123.70628	Halpin 52
H. hybrid 17d	Days gulch at Josephine Cr., Josephine Co., OR	N 42.22124 W 123.70629	Halpin 53
H. serpentinicola 20	South slope of $8 Mt., near Selma, Josephine Co., OR.	N 42°14.68 W 123°40.95'	Fishbein 5958
H. bracteosa 20	South slope of $8 Mt , Josephine Co.,	N 42°14.68' W	Fishbein 5969

	OR.	123°40.95'	
H. serpentinicola 21	Near Agness, Curry Co., OR.	N 42.55593 W124.09096	Halpin 31, 84
H. serpentinicola 22	Illinois River trail, Josephine Co., OR.	N 42.37823 W 123.82185	Halpin 39
H. serpentinicola 23	Illinois River trail, Josephine Co., OR.	N 42.37794 W 123.80520	Halpin 42
H. serpentinicola 24	Slate Creek, near Wonder, Josephine Co., OR.	N 42.37118 W 123.58439	Halpin 44
H. alba 25	Fish Hatchery Park, near Wilderville, Josephine Co., OR.	N 42.35999 W 123.42229	Halpin 45
H. serpentinicola 26	Free and Easy Pass Rd, near Kirby, Josephine Co., OR.	N 42.0214 W 123.67761	Halpin 47
H. alba 27	Near Kangaroo Lake, Siskiyou Co., CA.	N 41.36308 W 122.64088	Halpin 62
H. alba 28	Rock Fence Creek Botanical Area, Siskiyou Co., CA.	N 41.35099 W 122.62108	Halpin 63
H. alba 29	Mt Scott wilderness area, Siskiyou Co., CA.	N 41.27633 W 122.69845	Halpin 67
H. alba 30	Trinity Alps Creek, Trinity Co., CA.	N 40.88178 W 122.88097	Halpin 68
H. serpentinicola 31	Rouge River National Forest. Along S. side of Whisky Creek near bridge of Wimer/Lone Mt. road, Josephine Co., OR.	N 42.02250 W 123.77419	Halpin 72
H. sp. 32	Six Rivers National Forest. Along FS 18N07, about 12 miles east from CA 199, Del Norte Co., CA.	N 41.91221 W 123.66676	Halpin 76
H. alba 33	Along Happy Camp Rd/ County HWY 5828 at junction with NF 4804, Josephine Co., OR.	N 42.06412 W123.57381	Halpin 77
H. alba 34	Six Rivers NF. 0.5 air miles east of Chimney Rock, Siskiyou Co., CA.	N 41.58738 W 123.69275	Halpin 79
H. serpentinicola 35	Game Lake, Rogue-Siskiyou NF, Curry Co., OR.	N 42.43124 W 124.08376	Halpin 83
H. bracteosa 36a	Days Gulch tributary, near $8 mountain, Josephine Co., OR.	N 42.23559 W 123.69646	Halpin 23
H. bracteosa 36b	Days Gulch tributary, near $8 mountain, Josephine Co., OR.	N 42.23542 W 123.69731	Halpin 71
H. alba 37	Klamath NF. Along FS14N21, 0.4 mi NE of Junction with NF 14N17, Siskiyou Co., CA.	N 41.54958 W 123.61906	Halpin 78
H. serpentinicola 38	Near Vulcan Lake, Curry Co. OR.		Fishbein 6231
H. serpentinicola 39	Rock Creek, west of O'Brien, Josephine Co., OR.	N 42.03867 W 123.75439	Halpin 85
H. bracteosa 40	Star Flat, near Selma, Josephine Co., OR.	N 42.28038 W 123.67806	Halpin 24

H. bracteosa 41	Free and Easy Creek, Josephine Co., OR.	N 42.20301 W 123.68060	Halpin 46
H. atropurpurea 42	Lynholm Gulch, Josephine Co., OR.	N 42.203889 W 123.72694	Halpin 48
H. atropurpurea 43	Josephine Creek, Josephine Co., OR.	N 42.18855 W 123.71422	Halpin 66
H. atropurpurea 44	Woodcock Bog RNA. Alongside woodcock creek, Josephine Co., OR.	N 42.12809 W 123.69854	Halpin 70
Camassia howellii	Sexton Mountain, near Grants Pass, OR.	42° 35.675'N 123° 22.268'W	*Kephart 593* (WILLU)
Camassia leichtlinii	Popcorn Swale, near Glide, OR.	43° 18.079'N 123° 13.558'W	*Kotaich 105* (WILLU)
Camassia quamash	Donner Lake, near Truckee, CA.	39° 19.356'N 120° 14.811'W,	*Sultany s. n.* (WILLU)
Chlorogalum angustifolium	Near Gold Hill, OR.	N 40° 38'30.80", W122° 21'47.50"	Callahan (1) *s. n.* (OSC 216206)
Chlorogalum pomeridianum	8 Dollar Mountain, near Selma, OR.	42° 13.89'N 123° 39.06'W	Fishbein 5972 (OKLA)
Chlorogalum parviflorum	Near Encinitas, CA.	N 33.092 W 117.288	Sanders 30293 (UCR)
Chlorogalum purpureum	Near Jolon, CA.	N/A	Wilken 15701 (SBBG)
Hesperoyucca whipplei	Near Ejido Uruapan, Baja California.	31.59477˚N 116.42676˚W	Fishbein 6390 (OKLA)
Schoenolirion croceum	Near Burkeville, TX.	N/A	Singhurst 6557 (TEX)

Figure 6. Map of SW Oregon and NW California. The white dotted line represents the Oregon-California border. The map inset is of the western Illinois Valley of SW Oregon. Circles represent populations that are labeled by population number and colored by *Hastingsia* species identifications: *H. alba* are in orange; *H. serpentinicola* are yellow-green; *H. bracteosa* are lavender; *H. atropurpurea* are pink; populations with unknown identities are grey-green; one population (17) with multiple suspected hybrids is teal.

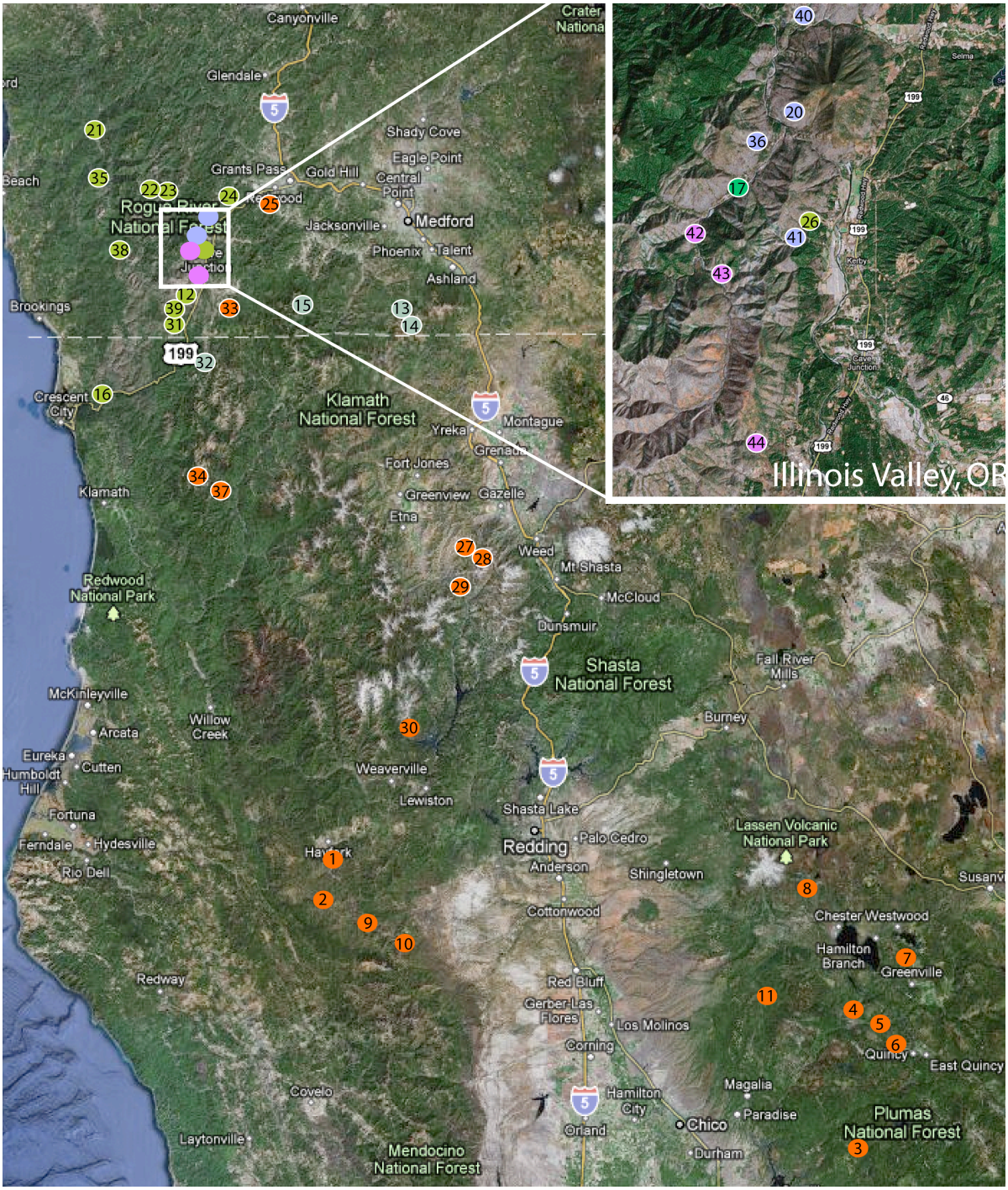

Table 9. Attributes of the aligned sequences of three plastid loci for all taxa including outgroups, with values for just the *Hastingsia* ingroup in parentheses.

Locus	Aligned length	Length after ambiguously aligned regions excluded	Variable sites included	Parsimony informative sites included
trnD-T	1165	1146	96 (24)	39 (8)
rpl16 intron	1361	1078	91 (28)	38 (14)
psbJ-petA	1303	1223	100 (29)	40 (15)
Total	3829	3447	287 (81)	117 (37)

Table 10. ML parameter estimates and Bayesian credible intervals for substitution models selected for use.

Partition	Model	T_i/T_v	r_{AC}	r_{AG}	r_{AT}	r_{CG}	r_{CT}	π_A	π_C	π_G	π_T	α
Bayesian analysis 95% credible interval of parameter summaries.												
trnD-trnT	GTR+Γ	-	0.043-0.147	0.197-0.370	0.026-0.091	0.050-0.191	0.186-0.357	0.287-0.338	0.162-0.205	0.156-0.198	0.301-0.352	0.017-0.716
rpl16 intron	HKY+Γ	1.73-3.78	-	-	-	-	-	0.360-0.414	0.132-0.173	0.168-0.212	0.245-0.295	0.036-129.46
psbJ-petA	GTR+Γ	-	0.077-0.203	0.187-0.357	0.033-0.097	0.008-0.118	0.232-0.417	0.313-0.364	0.136-0.175	0.142-0.181	0.319-0.369	0.018-0.676
Parameters for likelihood analysis in RAxML												
trnD-trnT	GTR+Γ	-	0.475	1.628	0.296	0.585	1.536	0.311	0.188	0.180	0.321	0.207
rpl16 intron	GTR+Γ	-	0.430	1.025	0.631	0.555	2.190	0.385	0.156	0.192	0.267	0.300
psbJ-petA	GTR+Γ	-	0.753	1.592	0.341	0.212	1.904	0.349	0.156	0.155	0.340	0.194
Parameters for likelihood analysis in Garli												
3-loci combined	GTR+Γ	-	0.616	1.531	0.454	0.530	2.049	0.35	0.16	0.17	0.31	0.220

Figure 7. Strict consensus of maximum parsimony cladograms produced by conventional heuristic and ratchet searches with nonparametric bootstrap percentages greater than 50% presented above branches. Clades A, B, and C represent the three major clades recovered in each analysis. SN refers to the clade of individuals from the Sierra Nevada.

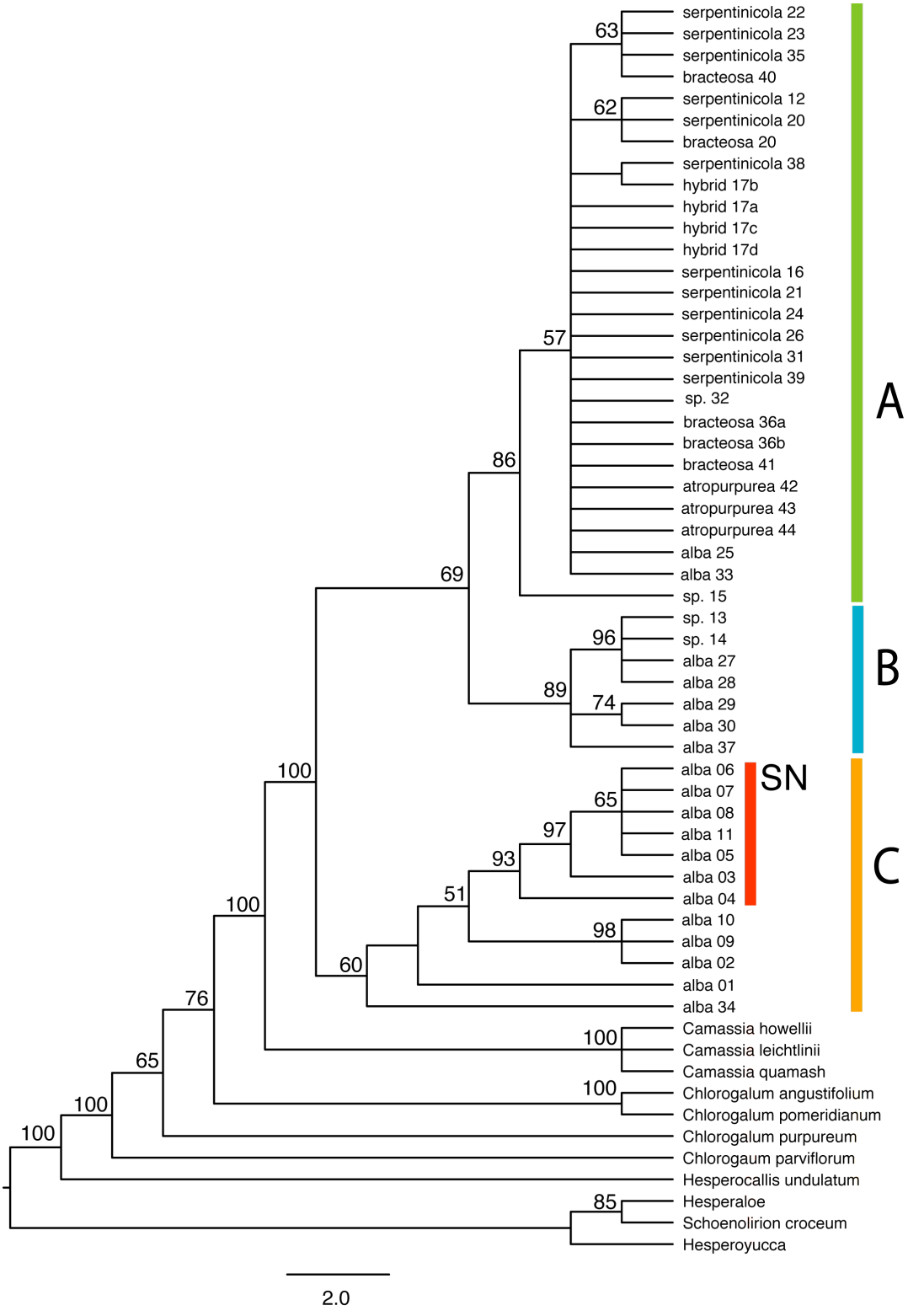

Figure 8. The maximum likelihood phylogram produced in RAxML. RAxML BS, GARLI BS, and Bayesian posterior probabilities are presented above the branches. Clades A, B, and C represent the three major clades recovered in each analysis. SN refers to the clade of individuals from the Sierra Nevada.

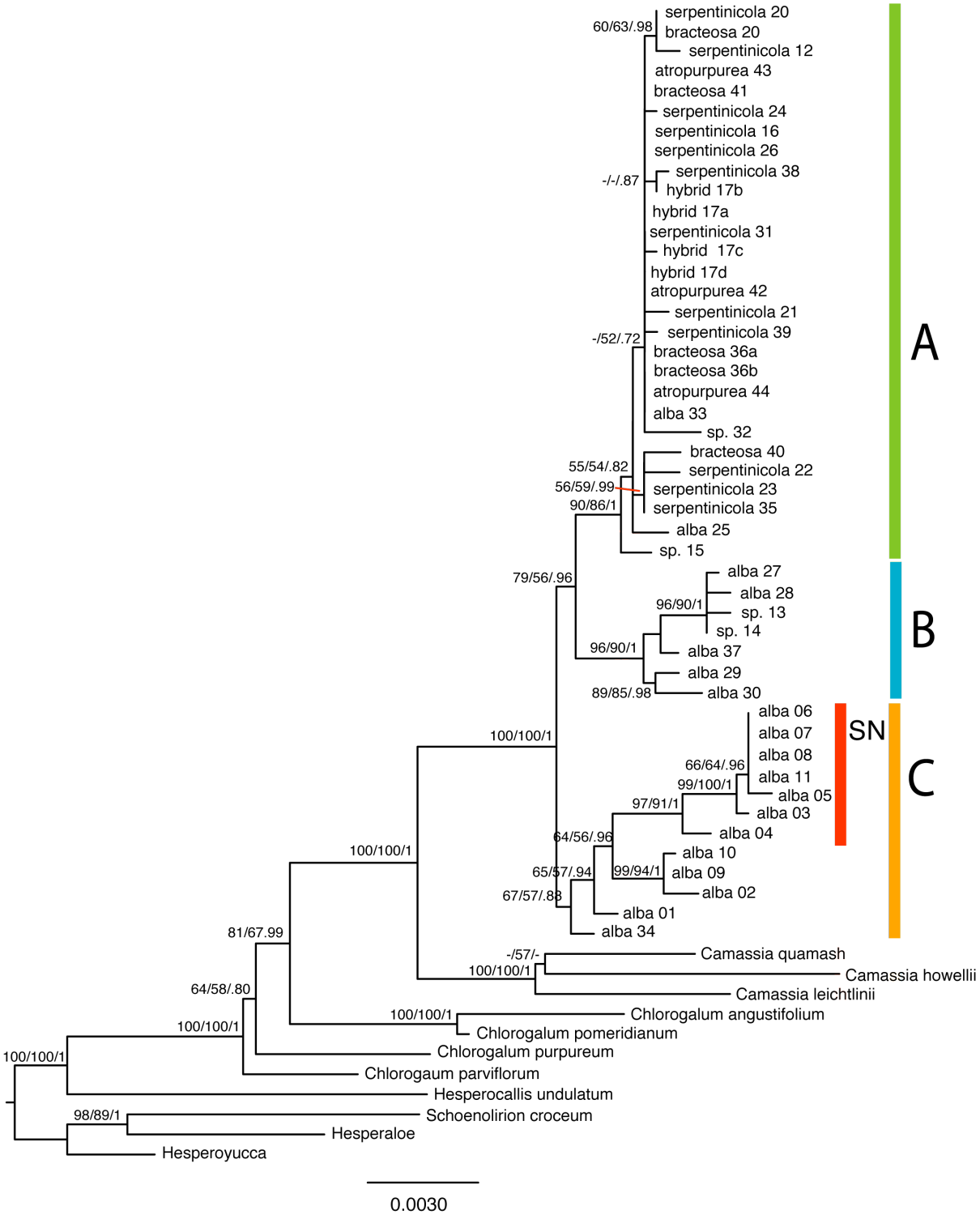

Figure 9. The four TCS haplotype networks recovered when the maximum connection limit was set to 8 steps. Each branch segment represents a point mutation, with each solid circle representing a hypothetical haplotype. Squares represent the most probable ancestral haplotype for each network. Green indicates distributions in the Illinois Valley and surrounding Siskiyou mountains, whereas the eastern Siskiyou and Klamath ranges are in blue, and the Sierra Nevada is in orange.

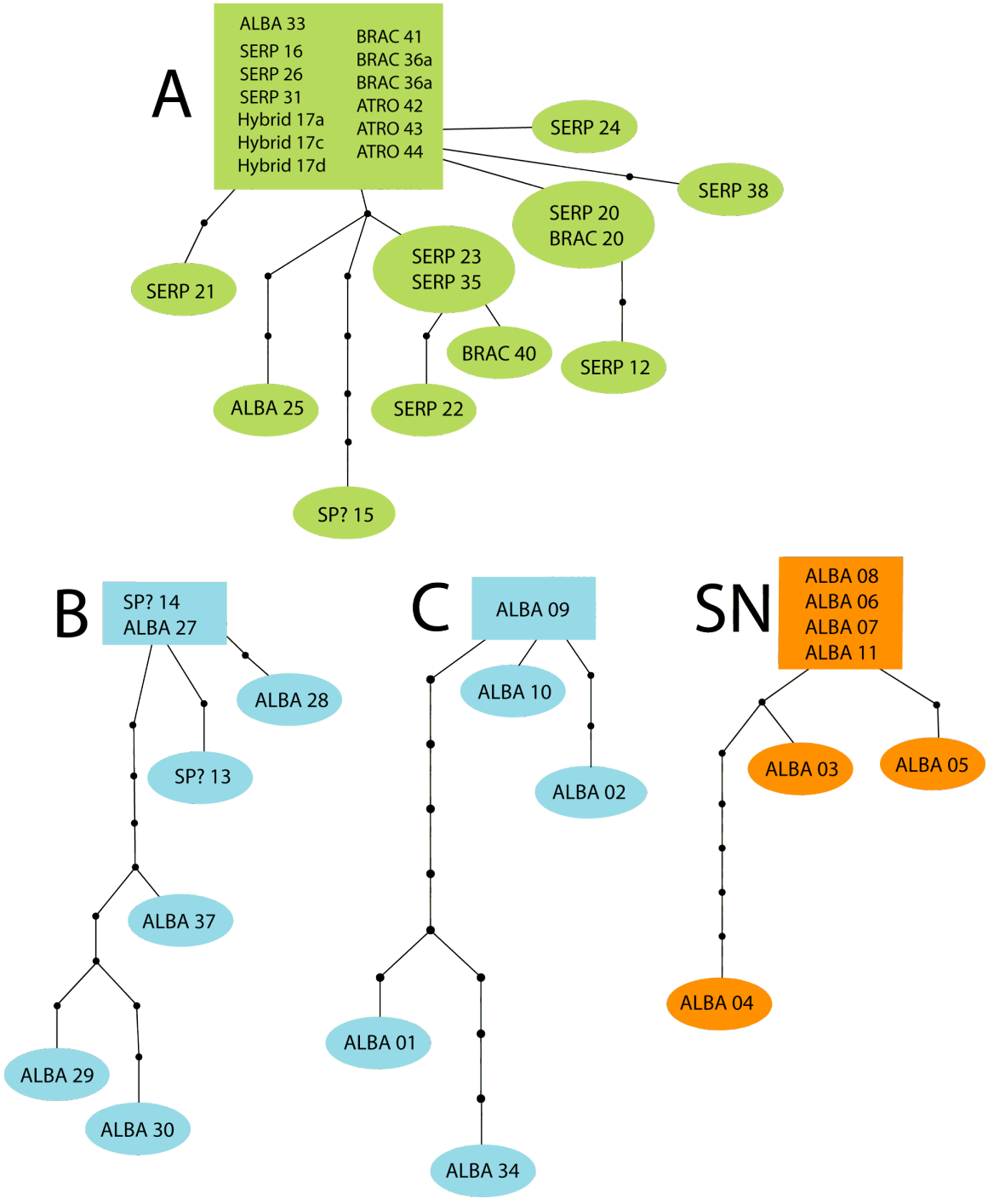

Table 11. Results of the parsimony based (Templeton and Winning-Sites) and the likelihood (SH) topology tests. Hypotheses 1-6 list the groups that were constrained to be monophyletic. Groups with multiple species were constrained as single clade, without constraining the monophyly of the individual species. * = $P < 0.05$.

Constrained searches testing the monophyly of:	Templeton: test statistic/P-value	Winning-sites: test statistic/P-value	SH: difference in –ln L/P-value
1a. *H. alba* – all samples	1.5/0.0280*	0.857/0.1250	33.0087/0.020*
1b. *H. alba* – all except *25, 33*	2.5/0.3173	0.750/0.6250	3.79590/0.226
2. *H. serpentinicola*	6/0.03387	50.500/1.0000	22.59583/0.051
3. *H. bracteosa*	0/0.1025	1.0/0.2500	21.61568/0.053
4. *H. atropurpurea*	0/1.000	1.0/1.0000	0.00000/0.496
5. *H. alba* and *H. serpentinicola*	18.5/0.0961	0.750/0.1460	44.51186/0.007*
6. *H. bracteosa* and *H. atropurpurea*	0/0.1025	1.0/0.2500	21.61568/0.053

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CHAPTER IV

CONCLUSIONS

Serpentine soils have been of great interest to ecologists and evolutionary biologists because they contain toxic concentrations of metals, giving rise to plant populations with specialized adaptations, but also with high levels of endemism and extreme rarity (Alexander 2007). Among the serpentine endemic and tolerant taxa of western North America are species of the closely related genera *Chlorogalum, Camassia*, and *Hastingsia*. Along with *Schoenolirion,* these genera comprise the subfamily Chlorogaloideae (sensu Speta of Hyacinthaceae; 1998). Phylogenetic analyses strongly support their placement in Agavaceae, however relationships within this group and their relationship within Agavaceae are largely unknown. Furthermore, a dispute on species circumscription within *Hastingsia*, ranging from two to four species, has prompted a more focused investigation at the population level. This study composed phylogenetic analyses based on chloroplast DNA to estimate these relationships and explore the evolution of serpentine tolerance among these genera.

The monophyly of Chlorogaloideae sensu Speta (i.e., *Chlorogalum, Camassia, Hastingsia,* and *Schoenolirion*) was not supported as monophyletic, with *Schoenolirion* found in a clade with *Hesperoyucca* and *Hesperaloe.* However, *Chlorogalum, Camassia*, and *Hastingsia* (core Chlorogaloideae) were highly supported as monophyletic, as was the sister relationship of *Hastingsia* and *Camassia.* The unexpected placement of *Schoenolirion* outside of

Chlorogaloideae necessitates further investigations of relationships among these genera and certainly warrants new circumscription of the subfamily. However, a new treatment the subfamily would be premature in this study because relationships between the core Chlorogaloideae and the remainder of Agavaceae are still uncertain. The placement of *Hesperocallis* was somewhat ambiguous and was either moderately supported as sister to the core Chlorogaloideae or unresolved in a polytomy with the core Chlorogaloideae and the *Schoenolirion, Hesperoyucca,* and *Hesperaloe* clade. Therefore, analyses including additional sequence data (i.e., nuclear loci) should be explored before making taxonomic revisions. The sister taxon of the core Chlorogaloideae would have important implications for the biogeography and evolution of morphology of these taxa. A sister relationship with *Hesperocallis* would imply an origin near southern California for the core Chlorogaloideae. It would also imply that the tunicate bulbs and herbaceous habit originated with the ancestor of these taxa, and that these characters in *Schoenolirion* are the result of convergent evolution. The relationships of *Schoenolirion*, *Hesperaloe*, and *Hesperoyucca* merits further investigation, since these taxa may be more genetically and morphologically similar than previously thought.

Within *Hastingsia*, none of the four species was supported as monophyletic, but the non-monophyly was not statistically supported. Low sequence divergence among three of the four species indicates that either these species have diverged recently enough that chloroplast DNA may not harbor enough genetic variation to recover these taxa as distinct lineages, or that limited gene flow is occurring among these taxa, or a combination of the two. However, three major clades were recovered in the genus corresponding to large geographical regions. The presence of well-supported clades within *H. alba* may be an indication of the effects of inhabiting isolated outcrops of serpentine soils. The naturally fragmented habitats that serpentine soils provide habitats are often small and geographically disjunct, which may foster lineage diversification and speciation through reduced gene flow among populations.

According to Anacker (2011), the effects of serpentine soils on plant speciation this is still one of major unanswered questions in the study of the evolution of serpentine endemics. Serpentine habitats can either promote speciation and lineage divergence though adaptive radiations as new taxa adapt to a previously unavailable niche, or serpentine soils may inhibit speciation and diversification because of their highly insular environments. Evidence for both scenarios exist throughout phylogenetic studies that include taxa occurring on serpentine. For example, within the large and diverse genus *Allium,* many species have independently adapted onto serpentine substrates, but there are also cases where serpentine tolerant species were inferred to have given rise to additional tolerant species (Nguyen et al. 2008). However, many phylogenies suggest independent origins for serpentine tolerance with no further indication that these species in turn gave rise to new serpentine tolerant species, including studies of the genera *Navarretia* (Spencer and Porter 1997) and *Layia* (Baldwin 2005). In other cases, there is evidence for both scenarios in a single genus (i.e., *Calochortus*; Patterson and Givnish 2004). Within the core Chlorogaloideae, an adaptation onto serpentine soils may have supported the diversification of entire genera such as *Chlorogalum* and *Hastingsia,* in which the majority of taxa occur on serpentine. However, it is unclear why *Camassia,* which occurs near a dense occurrence of serpentine outcrops in southern Oregon and northern California, only contains a single species that occurs on serpentine, and why *Hastingsia*, which consists entirely of serpentine endemic and tolerant taxa, has the fewest number of species among the core Chlorogaloideae genera. Are the consequences of adapting onto serpentine an opportunity for speciation in some taxa and limiting for others? Based on the studies mentioned above, it appears to be circumstantial. However, the vast number of serpentine endemic and tolerant taxa across all plant taxa suggests that the adaptation onto serpentine soils is easy to acquire, but will have a mixture of effects on the evolution of different taxa.

Additional studies on the core Chlorogaloideae will be necessary, not only to better understand currently unresolved relationships, but also to add to our knowledge of how species

respond and change over time to unique habitats such as serpentine outcrops and other similarly fragmented and harsh habitats that occur around the world. Furthermore, monocotyledons are particularly underrepresented among studies of serpentine taxa. The core Chlorogaloideae represents a model system for studying the evolution of adaptation onto serpentine soils at a wide range of taxonomic levels. This group of three closely related genera can provide information on how serpentine tolerant and endemic taxa evolve at the generic and species level, in both widespread and narrowly endemic taxa. I sincerely hope that this unique group will continue to be investigated in order to broaden our understandings of serpentine communities and the evolution of species in general.

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VITA

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Master of Science

Thesis: A CHLOROPLAST PHYLOGENY OF AGAVACEAE SUBFAMILY CHLOROGALOIDEAE WITH A FOCUS ON SPECIES RELATIONSHIPS IN HASTINGSIA

Major Field: Botany

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Education:

Completed the requirements for the Master of Science in Botany at Oklahoma State University, Stillwater, Oklahoma in May, 2011.

Completed the requirements for the Bachelor of Science in Biology at Portland State University, Portland, Oregon in 2008.

Experience: Herbarium Curatorial Assistant, Oklahoma State University, Department of Botany. I assisted in all aspects of maintaining the OSU Herbarium (OKLA), including specimen preparation, filing, transaction management, accessioning new collections, and databasing specimens from 2009 to 2010. Graduate Teaching Assistant, Portland State University, Department of Biology. Taught the lab portion of Principles of Biology and Plant Ecology from 2008 to 2009. Field and Lab Assistant, Portland State University, Lab of Dr. Sarah Eppley. Collection and identification of thermophilic mosses and associated species from Lassen National Volcanic Park, CA in 2007. Lab Assistant**,** Portland State University, Lab of Dr Ken Stedman. General maintenance and cleaning of lab, making stock media and maintaining *Sulfolobus* (Archaea) cultures from 2006 to 2007.

Professional Memberships: American Society of Plant Taxonomists, Botanical Society of America, California Native Plant Society, California Botanical Society, Native Plant Society of Oregon, Oklahoma Native Plant Society, Society of Systematic Biologists, Ecological Society of America, and American Society of Naturalists.

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Institution: Oklahoma State University Location: Stillwater, Oklahoma

Title of Study: A CHLOROPLAST PHYLOGENY OF AGAVACEAE SUBFAMILY CHLOROGALOIDEAE WITH A FOCUS ON SPECIES RELATIONSHIPS IN HASTINGSIA

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Scope and Method of Study: Serpentine soils contain toxic concentrations of metals, giving rise to plant populations with specialized adaptations, but also with high levels of endemism and extreme rarity. Among the serpentine endemic and tolerant taxa of western North America are species of the closely related genera *Chlorogalum, Camassia*, and *Hastingsia*. Along with *Schoenolirion,* these genera comprise the subfamily Chlorogaloideae (sensu Speta of Hyacinthaceae; 1998). Phylogenetic analyses strongly support their placement in Agavaceae, however relationships within this group and their relationship within Agavaceae are largely unknown. Furthermore, a dispute on species circumscription within *Hastingsia*, ranging from two to four species, has prompted a more focused investigation at the population level. This study composed phylogenetic analyses based on chloroplast DNA to estimate these relationships and explore the evolution of serpentine tolerance among these genera.

Findings and Conclusions: The monophyly of Chlorogaloideae (i.e., *Chlorogalum, Camassia, Hastingsia,* and *Schoenolirion*) was not supported as monophyletic, with *Schoenolirion* found in a clade with *Hesperoyucca* and *Hesperaloe.* However, *Chlorogalum, Camassia*, and *Hastingsia* (core Chlorogaloideae) were highly supported as monophyletic, as was the sister relationship of *Hastingsia* and *Camassia.* The placement of *Hesperocallis* was either moderately supported as sister to the core Chlorogaloideae or unresolved in with the core Chlorogaloideae and the *Schoenolirion, Hesperoyucca,* and *Hesperaloe* clade. The genera of Chlorogaloideae were each highly supported as monophyletic, but *Chlorogalum* was not, with two species placed outside of the rest of the core Chlorogaloideae. No species of *Hastingsia* was supported as monophyletic, but was not statistically supported. However, large-scale geographic structure within the genus was found, with three major clades recovered in the phylogeny corresponding to large geographical regions. Ancestral character reconstructions of serpentine tolerance in the core Chlorogaloideae suggested multiple origins for the adaptation onto serpentine soils.

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